Identifying the Source of Dioxin in Sediment from Furuayase River, Japan, Based on Specific Congener Profiles

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ABSTRACT
A specific dioxin composition was detected in some sediment samples collected from the highly polluted Furuayase River, Japan. The dominant tetrachlorodibenzofuran (TeCDF) congeners in the samples were 1,3,7,8- and 1,3,7,9-tetrachlorodibenzofuran (1,3,7,8-/1,3,7,9-TeCDF). To identify the sources of the pollution with the specific dioxin composition, the dioxin compositions were studied in samples of drainage discharge water, sewage discharge water, and sludge in the sewage works from four chemical factories located in Furuayase River drainage basin. The specific composition was detected in the sewage discharge water and the sludge in the sewage works of one factory. This factory had previously produced 2,4,6-trichlorophenylhydrazine (TCPH) as an intermediate in the synthesis of a photographic medicine. It was investigated whether dioxin impurities were present in commercial TCPH reagents and whether 1,3,7,8-/1,3,7,9-TeCDF by-products might be formed during manufacture of TCPH. The dioxin homologue and congener profiles from one commercial reagent were similar to those of the sewage discharge water and sludge in the sewage works from the aforementioned factory. Our results indicated a possible pathway for the generation of 1,3,7,9-TeCDF during manufacture of TCPH.

Keywords: 1,3,7,9-tetrachlorodibenzo-furan, pollution source, 2,4,6-trichlorophenylhydrazine, 2,2′,4,4′,6,6′-hexachlorobiphenyl (PCB 155)

INTRODUCTION
Dioxin-like compounds are still found in the Japanese environment even though these compounds have been strictly regulated in Japan for over ten years. Under Japanese law, dioxin is a comprehensive term including polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs). Dioxin has been released into the environment through combustion, metal smelting, bleaching, sterilization, agrochemical formulation and other industrial processes (US EPA, 2006). Another major source of dioxin is leakage of PCB and PCDFs from power distribution transformers. The PCB formulation, which was once commonly used as a coolant and insulation fluid in transformers, is a complex mixture of DL-PCBs (Noma et al., 2004), and PCDFs are formed when PCB is heated or oxidized (Buser et al., 1978).
Many organochlorine pesticides, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-nitrophenyl 2,4-dichlorophenyl ether (NIP), chlomethoxynil, pentachloronitrobenzene (PCNB), and pentachlorophenol (PCP), contain dioxins (US EPA, 2006; Holt et al., 2010). The herbicidal PCP and chlornitrophen (CNP) were frequently used in rice fields in Japan (Masunaga et al., 2001a), and these pesticides are potential sources of dioxins in sediments of Japanese lakes, ponds, and seas (Sakurai et al., 2000; Masunaga et al., 2001b; Okumura et al., 2004). As well as a pesticide, dioxazine dyes (Williams et al., 1992; US EPA, 2006), phthalocyanine dyes (Santl et al., 1994; US EPA, 2006), and chloranil-based dyes (Alcock and Jones, 1997) are additional sources of dioxins.

Dioxin released into the environment ultimately accumulates on sediment surfaces. Dioxins in sediments have attracted the attention of many researchers, and identifying the source of dioxin pollution has been a topic of major interest. To identify a source, multivariate statistical methods, such as factor analysis, cluster analysis, principal component analysis, and multiple regression analysis, have been used alone (e.g., Götz et al., 1998; Sundqvist et al., 2009) or in combination (e.g., Fiedler et al., 1996; Masunaga et al., 2001b). Receptor models, such as the chemical mass balance model (Su and Christensen, 1997; Sakai et al., 2008) and positive matrix factorization (Sundqvist et al., 2010; Takeda et al., 2011), have also been used to identify a source of dioxin pollution.

The above analyses are based on using a huge number of existing dioxin congeners. In contrast, using representative congeners, Ohtsuka et al. (2007) and Minomo et al. (2010) reported a simple method for estimating the toxic equivalent quantities (TEQs) from four major dioxin sources (combustion, PCB, PCP, and CNP). Kakimoto et al. (2006) investigated the homologue and isomer distributions of dioxins in lagoon water samples, and reported high concentrations of 1,3,6,7,8-pentachlorodibenzofuran (1,3,6,7,8-PeCDF) and 1,3,6,8-chlorine-substituted PCDFs in the samples. These non-toxic congeners were usually only minor constituents in environmental water samples, Kakimoto et al. (2006) successfully traced the source of these congeners back to a dye manufacturing plant.

The Japanese environmental quality standard for dioxin is sometimes exceeded in sediment samples collected from Furuayase River, which flows through the southeast part of Saitama Prefecture, Japan. Polluted sediment in Furuayase River is primarily distributed in an area close to Matsueshinbashi Bridge, with sediment upstream of the bridge containing a high level of 1,3,7,8-/1,3,7,9-TeCDF congeners (Hosono et al., 2012a). A drainage ditch passing through an industrial area and flowing into the upper polluted part of Furuayase River is the suspected source of the pollution. The composition of dioxins in drainage water sampled during a rain event was similar to that found in polluted sediment from the upstream area (Hosono et al., 2012b).

The aims of our research were to identify the source of dioxin in the sediment of Furuayase River and to estimate the source’s contribution to dioxin contamination in the sediment. We focused on the drainage discharge water, sewage discharge water, and sewage sludge from four chemical factories as potential sources of pollution, using the
specific dioxin congener profile in which 1,3,7,8-/1,3,7,9-TeCDF were dominant. In addition, we examined processes for generating the specific dioxin composition to propose a plausible pathway for the production of 1,3,7,9-TeCDF.

MATERIALS AND METHODS

Study area

Furuayase River is an urban river flowing through the southeastern part of Saitama Prefecture, Japan (Fig. 1). The river, which is only 5.4 km long, connects with Ayase River, one of the main rivers of Saitama Prefecture. Several drainage ditches flowing through an industrial area empty into Furuayase River. Matsueshinbashi Bridge is built over the downstream part of the river, and dioxin-polluted sediment is distributed both upstream and downstream of the bridge.

In an initial study in July 2005, we collected and analyzed samples of industrial wastewater discharged into the drainage ditch that connects with the Furuayase River 250 m upstream from the Matsueshinbashi Bridge. However, a sewerage system has since been constructed, and some of the industrial wastewater is now discharged into the sewerage system.

Fig. 1 - Location of Furuayase River. Dioxin concentrations in sediment exceed the Japanese environmental standard within the area 300 m upstream and 200 m downstream of Matsueshinbashi Bridge. The dashed line shows the drainage ditch connects with Furuayase River 250 m upstream of Matsueshinbashi Bridge. The shaded sections indicate an industrial area.
Sampling
We collected the samples from four factories located in the drainage basin. Factory A produces zinc chloride and polyelectrolytes. We sampled water flowing into the ditch (water A) and dehydration cakes (sludge A) from the sewage works of factory A. Factory B reprocesses waste organic solvents. We sampled water flowing into the ditch (water B) and sludge (sludge B) from the oil–water separation tank in factory B. Factory C manufactures pharmaceuticals, dye and pesticide intermediates. We sampled water flowing into the ditch (water C) from the sewage works of factory C. Factory D manufactures pharmaceutical and other intermediates. We collected two types of water samples (water D1 and water D2) and sludge (sludge D) from the sewage works of factory D. Water D1 flowed into the ditch and was composed mainly of indirect coolant, whereas water D2 flowed into sewers and consisted mainly of process water. In addition, we sampled the ditch sediment at a point where the discharge waters from factories B, C, and D met.

As described in the result and discussion section, we determined that 1,3,7,8-/1,3,7,9-TeCDF were the dominant TeCDF congeners in the samples of sewage discharge water and sewage sludge of factory D. These congeners were plausibly generated during production of 2,4,6-trichlorophenylhydrazine (TCPH) in factory D. Because factory D had already ceased producing TCPH at the time of our study, we were not able to determine if TCPH or any possible by-products from the synthesis of TCPH were present in the wastewater of factory D. Moreover, industrial-grade TCPH from other factories is difficult to obtain. Therefore we purchased TCPH reagents from three chemical companies (reagents 1 – 3) for analysis. The objectives of the analysis were to determine if dioxin impurities were present in the reagents and if 1,3,7,8-/1,3,7,9-TeCDF by-products were unintentionally generated during the manufacture of TCPH. However, because reagent-grade TCPH and industrial-grade TCPH are likely synthesized by different routes, the nature and concentration of impurities in reagent-grade TCPH may be different from those in industrial-grade TCPH.

Sample preparation
The standards of PCDD/PCDF/DL-PCB and 13C12-labeled PCDD/PCDF/DL-PCB were obtained from Wellington Laboratories, Ontario, Canada. All solvents used were purchased from Kanto Chemical, Tokyo, Japan. Hydrochloric acid (2 and 6 mol/L) and concentrated sulfuric acid were super special grade (Wako Pure Chemical Industries Ltd., Osaka, Japan). Wakogel DX, 44% sulfuric acid-impregnated silica gel, 10% silver nitrate-impregnated silica gel (Wako Pure Chemical Industries Ltd., Osaka, Japan), and active carbon dispersed silica gel (Kanto Chemical, Tokyo, Japan) were used for cleanup. The glass fiber filter, GA-55 (0.6-μm reservation particle size, 90-mm diameter) and the glass fiber extraction thimble, No. 86R (Toyo Roshi Kaisha, Tokyo, Japan) were used after heat treatment at 400°C for 3 h.

Water samples A, B, C, D1, and D2 were prepared according to the Japanese standard procedure for dioxin analysis (Japanese Standard Association, 1999). The sample pH was adjusted to 2 – 3 with 6 mol/L hydrochloric acid, and the 13C12-labeled PCDD/PCDF/DL-PCB cleanup spikes (17 2,3,7,8-chlorine substituted PCDD/Fs and 12 DL-PCBs) were added to the sample (250 pg for the tetra- to hepta-CDDs/CDFs and
DL-PCBs; 500 pg for octa-CDD/CDF). The spiked sample was filtered through a GA-55 glass fiber filter, and the filter residue was allowed to air-dry. The filter residue underwent Soxhlet extraction with toluene for 24 h, and the filtrate was subjected to liquid-liquid extraction with dichloromethane. The Soxhlet extract was combined with the liquid-liquid extract and concentrated by rotary evaporation, and the solvent was changed to hexane.

Sludge samples A, B, and D and the ditch sediment sample were prepared according to the Japanese standard procedure for determining dioxins in sediment (Environment Agency, Government of Japan, 2000). Air-dried sample (10 – 30 g) was placed in a No.86R glass fiber extraction thimble, and dioxins were obtained through Soxhlet extraction with toluene for 24 h. Cleanup spike (500 pg for the tetra- to hepta-CDDs/CDFs and DL-PCBs; 1000 pg for octa-CDD/CDF) was added to a portion of the extract, the spiked extract was concentrated by rotary evaporation, and the solvent was changed to hexane.

Each TCPH reagent (about 1 g) weighed to a precision of ± 0.01 mg was dissolved in 250 mL of ethanol, and then the cleanup spike (250 pg for the tetra- to hepta-CDDs/CDFs and DL-PCBs; 500 pg for octa-CDD/CDF) was added. The spiked solution was added to 250 mL of 2 mol/L hydrochloric acid, and this solution was shaken with 200 mL of hexane to extract dioxins. This extraction procedure was carried out three times. The combined hexane extract was dehydrated with sodium anhydride, concentrated by rotary evaporation, and subjected to a cleanup procedure.

The cleanup procedure was almost according to the Japanese standard procedure for dioxin analysis. The extracts were treated with concentrated sulfuric acid and 44% sulfuric acid-impregnated silica gel until the silica gel became colorless. The treated extracts were then purified by passing them through 10% silver nitrate-impregnated silica gel (3 g of packing, eluted with 100 mL of hexane) and active carbon dispersed silica gel (1 g of packing washed with 50 mL of hexane, eluted with 250 mL of hexane). The toluene eluates were spiked with the $^{13}$C$_{12}$-labeled internal standard (250 pg for the water and reagent samples; 500 pg for the sludge and sediment samples) and concentrated to 25 µL (water and reagent samples) or 50 µL (sludge and sediment samples).

**GC/MS analyses**

After cleanup, PCDD/Fs and DL-PCBs in the extracts were analyzed by high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS) using a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) and a JEOL JMS-700 mass spectrometer (JEOL, Tokyo, Japan). A CP-Sil 88 capillary column (60 m × 0.25 mm i.d., $d_l = 0.10 \mu m$; Varian, Lake Forest, CA, USA) was used for the analysis of tetra- to hexa-CDDs/CDFs, and a DB-5MS capillary column (60 m × 0.25 mm i.d., $d_l = 0.25 \mu m$; Agilent Technologies) was used for the analysis of hepta- to octa-CDDs/CDFs and DL-PCBs. Helium at a constant flow rate of 1.2 mL/min was the carrier gas. The mass spectrometer was operated in the positive-ion mode. Mass drift correction was performed using perfluorokerosene to provide a lock mass at a resolution of 10,000 or more. Recoveries of cleanup spikes were between 70%
and 95%. Differences between duplicate measurements of TEQ were less than 10% on the average.

Response factors for each congener were calculated relative to $^{13}$C$_{12}$-labeled homologues added to extracts as cleanup spikes. These response factors were used to quantify the native 2,3,7,8-chlorine-substituted congener for PCDD/Fs and DL-PCBs. The TEQs were calculated using the toxic equivalency factors reported by WHO/IPCS (Van den Berg et al., 1998).

RESULTS AND DISCUSSION

Dioxins in water, sludge and sediment samples

Dioxin concentrations in the drainage discharge water samples (water A, B, C, and D1) and the sewage discharge water sample (water D2) are listed in Table 1. Dioxin concentrations in the discharge water samples complied with the Japanese environmental standard for water (1 pg-TEQ/L). The dioxin concentration in water D2 was slightly over the Japanese environmental standard for water but below the Japanese wastewater standard (10 pg-TEQ/L). The PCDD/F homologue profiles of the drainage discharge water samples (waters A to D1) were similar; OCDD was dominant and accounted for 52% (water D1) to 61% (water A) of the total PCDD/Fs. The TeCDFs in water D1 accounted for 17% and was higher than that of the other water samples (5.5% – 5.7%). In contrast, the PCDD/F homologue profile of water D2 was clearly different from those of the other discharge water samples, including water D1 from the same factory; TeCDFs were dominant in water D2. Hosono et al. (2012b) found a characteristic correlation between the percentage of 1,3,7,8-/1,3,7,9-TeCDF to total TeCDFs and the percentage of total TeCDFs to PCDD/Fs in a dioxin investigation of drainage ditch water during a rain event. According to this correlation, the percentage of 1,3,7,8-/1,3,7,9-TeCDF to total TeCDFs should be high in water D2, in which TeCDFs accounted for 76% of the PCDD/F homologue composition. The percentage of 2,3,7,8-chlorine-substituted congeners in PCDD/Fs was as low as 7.1% for water D2 but ranged from 57% (water D1) to 69% (water A) for drainage discharge water samples. This small percentage of 2,3,7,8-chlorine-substituted congeners in PCDD/Fs for water D2 was the reason why water D2 had a relatively small TEQ value in spite of its high PCDD/F concentration.

Table 1 lists dioxin concentrations in the sludge samples from the sewage works (sludge A, B, and D) and in the drainage ditch sediment sample. Except for sludge B, dioxin concentrations in the samples exceeded the Japanese environmental standard for sediment (150 pg-TEQ/g). Low-chlorinated PCDFs were dominant in the homologue profile of sludge D, and TeCDFs accounted for 84% of the total PCDD/Fs. This fact coincides with the aforementioned correlation that the percentage of 1,3,7,8-/1,3,7,9-TeCDF to total TeCDFs was high in sludge D, which had a high TeCDFs to PCDD/Fs ratio. A similarly high percentage of 1,3,7,8-/1,3,7,9-TeCDF to total TeCDFs was found in the ditch sediment sample, in which TeCDFs accounted for 40% of the total PCDD/Fs. Because factory D discharged process water directly into the ditch, the high percentage of TeCDFs in the sediment sample was due to process water from factory D. The percentage of 2,3,7,8-chlorine-substituted congeners in PCDD/Fs was 3.3% for sludge D, 40% and 59% for sludges A and B, respectively, and 26% for
the ditch sediment. This small percentage of 2,3,7,8-chlorine-substituted congeners in PCDD/Fs for sludge D was the reason why sludge D had an extremely small TEQ value in spite of its high concentration of PCDD/Fs.

Table 1 - PCDD/F and DL-PCB concentrations and TEQ value in the drainage and sewage discharge water samples, sludge and drainage ditch sediment samples, and 2,4,6-trichlorophenylhydrazine reagents.

<table>
<thead>
<tr>
<th>Homologue/Congener</th>
<th>Drainage discharge water A</th>
<th>Drainage discharge water B</th>
<th>Drainage discharge water C</th>
<th>Drainage discharge water D</th>
<th>Sewage discharge water A</th>
<th>Sewage discharge water B</th>
<th>Sewage discharge water C</th>
<th>Sewage discharge water D</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Conc</td>
<td>%</td>
<td>Conc</td>
<td>%</td>
<td>Conc</td>
<td>%</td>
<td>Conc</td>
<td>%</td>
</tr>
<tr>
<td>PCDD/Fs</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>2,3,7,8-TCDD</td>
<td>0.16</td>
<td></td>
<td>&lt; 0.03</td>
<td></td>
<td>(0.03)</td>
<td></td>
<td>(0.04)</td>
<td></td>
</tr>
<tr>
<td>1,2,3,7,8-PoCDD</td>
<td></td>
<td></td>
<td>&lt; 0.03</td>
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<td></td>
<td></td>
<td>0.07</td>
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<td>1,2,3,7,8-HxCDD</td>
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<td>0.38</td>
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<td></td>
<td></td>
<td>0.34</td>
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<td>OCDD</td>
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<td>84</td>
<td>110</td>
<td>99</td>
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<td>2,3,7,8-TeCDF</td>
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<tr>
<td>1,2,3,7,8-PoCDF</td>
<td>(0.14)</td>
<td></td>
<td>0.31</td>
<td></td>
<td>0.52</td>
<td></td>
<td>17</td>
<td></td>
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<tr>
<td>2,3,4,7,8-PoCDF</td>
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<td></td>
<td>0.32</td>
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<td></td>
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<td>&lt; 0.06</td>
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<td>0.37</td>
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<tr>
<td>2,3,7,8-PCDD/Fl</td>
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<td>65</td>
<td>96</td>
<td>120</td>
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<td>0.55</td>
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<td>10</td>
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<td>0.52</td>
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<td>2.7</td>
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<td>(0.06)</td>
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<td>(0.05)</td>
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<tr>
<td>2,3',3',4,4'-PeCB (1015)</td>
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<td></td>
<td>21</td>
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<td>1.7</td>
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<td>18</td>
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<td>27</td>
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<td>48</td>
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<td>460</td>
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<td></td>
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<td>8.9</td>
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<td>0.85</td>
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<td>0.035</td>
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<td>0.064</td>
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<td>0.39</td>
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<tr>
<td>Total TEQ</td>
<td>0.54</td>
<td></td>
<td>0.33</td>
<td></td>
<td>0.93</td>
<td></td>
<td>0.71</td>
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</tr>
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</table>

a Dioxin concentrations are given in units of pg/L for water sample, pg/g for others. The values in the parentheses show less than lower quantitation limit.

b The numbers are percentages relative to total PCDD/Fs.

c Coelute with 1,2,3,4,8-PoCDF.

d Coelute with 1,2,3,4,7,9-HxCDF.

e TEQ values were calculated according to the 1998 TEF values established by the World Health Organization (Van den Berg et al., 1998)
The concentrations of TeCDFs and DL-PCBs were specifically high in the sewage discharge water and sludge samples of factory D. The same trend was found in sediment collected near the drainage water discharge point of factory D. Takeda et al. (2011) proposed an unknown source for dioxin pollution in the sediment of Furuayase River by applying a positive matrix factorization method. The homologue composition in our samples accorded with the characteristics of the proposed unknown source in Takeda’s report.

### Dioxins in TCPH reagents

The dioxin concentrations in TCPH reagents 1, 2, and 3 were 5,300, 3,500, and 46,000 pg/g, respectively, and the TeCDFs concentration accounted for more than 86% of the total PCDD/Fs concentration in all three reagents (Table 1). Detected 2,3,7,8-chlorine-
substituted congeners were limited; 1,2,3,4,7,8-HxCDF in reagents 1 and 3, and 1,2,3,7,8,9-HxCDF in reagent 2, and their concentrations were not so high. Therefore, the TEQ concentrations of dioxin in reagents 1, 2, and 3 were low (31, 4.6, and 30 pg-TEQ/g, respectively) and equal to the levels in soil and sediment samples generally found in the Japanese environment. However, we cannot exclude the possibility that a large amount of dioxins was discharged in wastewater from manufacturing and industrial processes, because the low values in reagents 1 – 3 were obtained for purified reagents. In fact, the concentration of TeCDFs in sludge D reached 210,000 pg/g, which is 5 – 60 times the values detected in the TCPH reagents.

The concentrations of DL-PCBs were less than 400 pg/g and lower than the concentrations of PCDD/Fs in the TCPH reagents. Therefore, the high concentrations of DL-PCBs in water D2 and sludge D did not reflect discharge from factory D of a by-product from the manufacture of TCPH, but rather contamination from other processes.

**Percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs**

A high percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs was found in the sediment of Furuayase River (Hosono et al., 2012a) and in the drainage water that flowed into the upper polluted part of the river during a rain event (Hosono et al., 2012b). The specific composition should contain information about the origin of the dioxin pollution. Therefore, we calculated the percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs for the discharge water, sludge, and sediment samples as well as the TCPH reagents (Table 2).

### Table 2 - 1,3,7,8-/1,3,7,9-TeCDF concentrations and the percentages of 1,3,7,8-/1,3,7,9-TeCDF to TeCDF in the water, sludge, drainage ditch sediment samples and 2,4,6-trichlorophenylhydrazine reagents.

<table>
<thead>
<tr>
<th></th>
<th>1,3,7,8-/1,3,7,9-TeCDF</th>
<th>TeCDFs</th>
<th>1,3,7,8-/1,3,7,9-TeCDF to TeCDFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water A</td>
<td>0.14</td>
<td>7.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Water B</td>
<td>0.81</td>
<td>5.7</td>
<td>14</td>
</tr>
<tr>
<td>Water C</td>
<td>0.88</td>
<td>8.4</td>
<td>10</td>
</tr>
<tr>
<td>Water D1</td>
<td>20</td>
<td>35</td>
<td>57</td>
</tr>
<tr>
<td>Water D2</td>
<td>1,100</td>
<td>1,600</td>
<td>70</td>
</tr>
<tr>
<td>Sludge A</td>
<td>80</td>
<td>2,000</td>
<td>4.0</td>
</tr>
<tr>
<td>Sludge B</td>
<td>56</td>
<td>450</td>
<td>13</td>
</tr>
<tr>
<td>Sludge D</td>
<td>180,000</td>
<td>210,000</td>
<td>87</td>
</tr>
<tr>
<td>Drainage ditch sediment</td>
<td>6,700</td>
<td>12,000</td>
<td>56</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>3,500</td>
<td>4,300</td>
<td>81</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>1,600</td>
<td>3,200</td>
<td>50</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>13,000</td>
<td>4,5000</td>
<td>29</td>
</tr>
</tbody>
</table>

*Units are pg/L for water samples, and pg/g for sludge, sediment, and reagent samples.*

*The numbers are percentages relative to total TeCDFs.*

*The discharge water from factories B, C, and D flowed into the ditch.*
The percentages of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs in water A, B, and C and sludge A and B were lower than those reported for sediment collected downstream of the discharge point of the drainage ditch (32% – 58%) and those for drainage ditch water during a rain event (25% – 43%). In contrast, we found the high percentages of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs in water D1 and D2, sludge D, and ditch sediment near factory D, and these values exceeded the ranges reported for sediment collected downstream of the discharge point of the drainage ditch. Because these samples were from wastewater from factory D or from sediment or sludge contaminated by wastewater from factory D, we surmised that factory D was the source of the dioxins with the high percentages of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs. However, factory D had never produced or used 1,3,7,8-/1,3,7,9-TeCDF in its industrial processes, and TCPH was the only chlorinated organic compound produced in factory D. Therefore, 1,3,7,8-/1,3,7,9-TeCDF were formed during a manufacturing process of TCPH in factory D.

We then investigated the relationship between 1,3,7,8-/1,3,7,9-TeCDF and TCPH. Because we could not obtain TCPH from factory D (factory D had ceased producing TCPH before we began our study), we determined the percentages of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs for reagents 1, 2, and 3; the percentages were 81%, 50%, and 29%, respectively (Table 2). There was a large variation of TeCDF congeners among the reagents, but we specifically detected 1,3,7,8-/1,3,7,9-TeCDF in all reagent samples, in reagent 1 in particular. Therefore, we concluded that the specific compositions of the factory D samples were the result of wastewater discharged during the manufacture of TCPH. The percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs for reagent 1 was higher than the percentages for water D2 and sludge D.

Using the concentration of 1,3,7,8-/1,3,7,9-TeCDF and TEQ detected in reagent 1 (3,500 pg/g and 31 pg-TEQ/g, respectively), we estimated the contribution of dioxins generated from the manufacture of TCPH to the sediment of Furuayase River. Assuming that all 1,3,7,8-/1,3,7,9-TeCDF in the sediment (31,000 pg/g at maximum, with an average of 3,200 pg/g) originated from TCPH, the contribution of dioxins from TCPH was 270 pg-TEQ/g at maximum, with an average value of 29 pg-TEQ/g, corresponding to 14% of TEQ in sediment. Some samples were strongly affected by TCPH since they had a high percentage (> 50%) of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs (Hosono et al., 2012a). Even in those samples, the contribution from dioxin due to TCPH was estimated to be 28 pg-TEQ/g (11% of TEQ of those sediment samples) on the average. The influence of TCPH on the TEQ of the sediment in Furuayase River was minimal. Therefore, we estimated contribution ratios of TEQs in sediment samples from four main dioxin sources using four indicator congeners (Ohtsuka et al., 2007). The contributions of TEQ to total TEQ were more than 70% originating from combustion, less than 20% from herbicides (PCP and CNP formulation), and about 10% from PCB on the average.

**Pathway for production of dioxins**

Generally, phenylhydrazines (including TCPH) are produced by the following process: An aniline is diazotized with sodium nitrite in the presence of hydrochloric acid to produce the corresponding diazonium chloride as an intermediate. Then, phenylhydrazine is synthesized by hydrogenation of diazonium chloride with sodium
sulfite in the presence of sodium hydroxide. The phenyldiazonium salt is highly reactive and is used to synthesize various compounds via the Sandmeyer reaction and diazo coupling reactions. In addition, the phenyldiazonium salt causes denitrification and aryl-aryl coupling in the presence of transition metals or via the Gomberg–Bachmann reaction under alkaline conditions. Thus, we propose the following plausible pathway to produce 1,3,7,9-TeCDF as a by-product during TCPH synthesis: First, PCB 155 is formed by aryl-aryl coupling of 2,4,6-trichlorobenzenediazonium chloride and then PCB 155 is oxidized to produce 1,3,7,9-TeCDF (Fig. 2).

As shown in Fig. 2, PCB 155 is a key compound in our proposed pathway, so we conducted experiments to ascertain the existence of PCB 155 in reagent 1, water D2, and sludge D, which contained high concentrations of 1,3,7,8-/1,3,7,9-TeCDF. We measured the PCB in the first fraction (hexane elute) through an active carbon column of these samples, using an HT8-PCB capillary column (60 m × 0.25 mm i.d.; SGE Analytical Science, Ringwood, VIC, Australia). Figure 3 shows the mass chromatograms of HxCB in these samples. In the mass chromatograms, PCB 155 was dominant in all these samples, but of low level or absent in incineration gas and commercial PCB formulations (Kim et al., 2004).

Under the previously described experimental conditions, 1,3,7,8- and 1,3,7,9-TeCDF coeluted on the CP-Sil 88 capillary column, which is commonly used for the analysis of tetra- to hexaCDD/Fs. To distinguish the congeners, we conducted an additional experiment using a DB-17 capillary column (30 m × 0.25 mm i.d., df = 0.25 µm, Agilent Technologies) and found that 1,3,7,9-TeCDF accounted for the most of the coeluting peak.

Fig. 2 - Plausible pathway for the generation of 1,3,7,9-tetrachlorodibenzofuran (1,3,7,9-TeCDF) by-product in the synthesis of 2,4,6-trichlorophenyl hydrazine (TCPH). The solid arrows show the pathway for the production of TCPH without by-products; the dashed arrows show the pathway for the generation of 1,3,7,9-TeCDF.
Fig. 3 - Mass chromatograms of HxCBs in reagent 1 (a), water D2 (b), and sludge D (c). A peak of PCB 155 was dominant and other peaks were negligible.

These results support our proposed pathway that 1,3,7,9-TeCDF was generated by the oxidization of PCB 155 which formed via aryl-aryl coupling of 2,4,6-trichlorobenzene diazonium chloride. Phenylhydrazine derivatives are still widely used during the manufacture of pharmaceuticals, agrochemicals, and dyes as well as other products. The possibility remains that dioxin may be formed unintentionally during these manufacturing processes and released to the environment. The approach described in this work will be useful for determining the fate and transport of dioxins in the environment.

CONCLUSIONS
Dioxin pollutants in sediment of Furuayase River had a specific, characteristically high percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs. Similar percentages were found in water samples collected during a rain event from a drainage ditch flowing into the upper polluted part of the river. Using specific composition as a tracer, the source of dioxin pollution was identified by measuring dioxin concentrations in the drainage discharge water, sewage discharge water, and sludge from the sewage works of four chemical factories located in Furuayase River drainage basin.
A dioxin composition similar to those of the samples from Furuayase River was found in the sewage discharge water and the sludge from the sewage works of one factory. In this factory, TCPH was previously produced as an intermediate in the synthesis of a photographic medicine. By-products produced during the manufacture of TCPH could be the source of the 1,3,7,8-/1,3,7,9-TeCDF pollutants in environmental samples, and we proposed a plausible pathway for their production during manufacture of TCPH. We purchased TCPH reagents from three chemical companies and determined if these reagents contained dioxin impurities, specifically 1,3,7,8-/1,3,7,9-TeCDF. One reagent had a high percentage of 1,3,7,8-/1,3,7,9-TeCDF to TeCDFs, confirming that dioxins of specific composition can be generated during the manufacture of TCPH.

We conducted experiments supporting our proposal that 1,3,7,9-TeCDF is generated as a by-product during synthesis of TCPH by hydrogenation of 2,4,6-trichlorobenzenediazonium chloride. In our proposed pathway, aryl–aryl coupling of the diazonium chloride generates PCB 155 which is then selectively oxidized to 1,3,7,9-TeCDF. The key compound PCB 155 was detected at high concentration not only in TCPH reagents but also in the sewage discharge water and sludge from the sewage works of this factory. Another experiment showed that 1,3,7,9-TeCDF was the major congener of the 1,3,7,8-/1,3,7,9-TeCDF pair. These results show that the 1,3,7,8-/1,3,7,9-TeCDF found in environmental samples was due to congeners produced in this factory through TCPH synthesis.

REFERENCES


