DETERMINATION OF TRICLOSAN, ITS CHLORINATED DERIVATIVES, AND THEIR METHOXYLATED ANALOGUES IN BIOTA

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Abstract
Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether: TCS) is an antimicrobial agent used widely for various consumer and personal care products, and it is often detected in aquatic environment. While, the information on environmental contamination of its transformation products such as methyl triclosan (MeO-TCS) or chlorinated derivatives 2',3,4,4'-tetrachloro-2-hydroxydiphenyl ether (TeCS I), 2',4,4',5-tetrachloro-2-hydroxydiphenyl ether (TeCS II), and 2',3,4,4',5-pentachloro-2-hydroxydiphenyl ether (PeCS), are very limited. In this study, the concentrations of TCS and its related compounds in the tissue of coastal fish (mullet), freshwater fish (crucian carp, largemouth bass), and aquatic bird (grey heron) were determined. The concentrations of TCS in freshwater fish collected from the river in urban area were obviously higher than those of coastal fish. The residue level of TCS in aquatic bird was relatively lower than freshwater fish. The three chlorinated derivatives were also found in freshwater fish and bird samples, although those levels were considerably lower than TCS. The levels of MeO-TCS were higher than TCS in fish, whereas the opposite result was observed in bird. Furthermore, the methoxylated analogues of the three chlorinated derivatives were detected in all samples. The levels of these methoxy analogues were the similar or higher compared to those of each hydroxy ones.

Introduction

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether: TCS) is an antimicrobial agent used widely for various consumer and personal care products such as soaps, toothpastes, deodorants, and fabrics. On the other hand, there is a growing concern about the occurrence and effects of TCS in the environment. TCS is acutely toxic to aquatic organisms [1] and recently it has been reported that exposure to low levels of TCS can disrupt the thyroid hormone-associated gene expression and can alter the rate of thyroid hormone-mediated postembryomic anuran development [2]. TCS has been often detected in aquatic environment such as river surface water and municipal wastewater treatment effluent [1, 3-5], and also residue in freshwater fish [6-8] and algae [5] were found. Meanwhile, a biotransformation product methyl triclosan (MeO-TCS) is more stable and persistent in the environment [3]. The occurrence of MeO-TCS was first reported for aquatic biota samples from Tokyo, Japan in 1984 [9]. Since then, it has been found in freshwater fish from Switzerland [10], German [7], and North America [8] in recent years. Besides, another transformation product chlorinated derivatives of TCS, namely TeCS Ⅰ, TeCS Ⅱ, and PeCS as shown in Figure 1, have been indicated to be unintentionally formed during the chlorine bleaching and sterilizing processes [11-13]. However, the contamination state of these compounds in the environment has not been elucidated yet. It is suspected that highly chlorinated derivatives of TCS and their methoxylated analogues (MeO-TeCS Ⅰ, MeO-TeCS Ⅱ, and MeO-PeCS) would be more lipophilic with potential for bioaccumulation. In the present study, we developed the simultaneous analytical method for TCS and its related compounds in biota sample, and determined the residue concentrations in some samples of wild fish and bird from Japan.

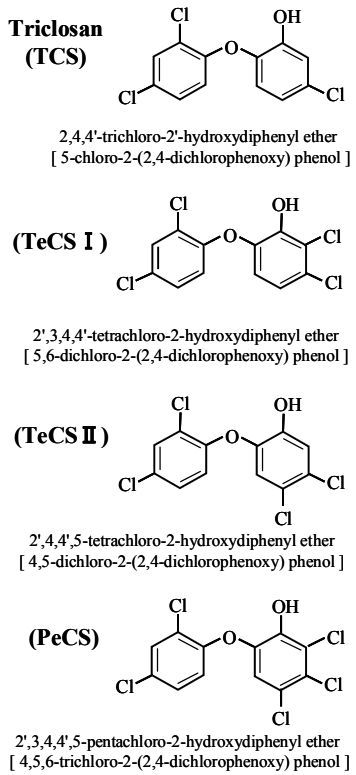

Fig. 1. Chemical structures of triclosan and its chlorinated derivatives. Their methoxylated analogues are substituted OCH_3 group to replace OH group.

Materials and Methods

Analytical standards A native TCS and $^{13}C_{12}$-labeled TCS were purchased from Wako Pure Chemicals Co. (Osaka, Japan) and Wellington Laboratories (Ontario, Canada), respectively. $^{13}C_{12}$-labeled MeO-TCS was prepared from $^{13}C_{12}$-labeled TCS by methylation with trimethylsilyl-diazomethane (Tokyo Chemical Industry Co., Tokyo, Japan). Chlorinated derivatives of TCS were synthesized by Dr. A. Kanetoshi, Hokkaido Institute of Public Health, Hokkaido, Japan, and provided from Dr. T. Okumura, Environmental Pollution Control Center, Osaka, Japan. TCS formulation manufactured by Ciba Specialty Chemicals, Irgasan® DP300 was obtained from Toyota Tsusho Corporation (Osaka, Japan).

Sample collections Coastal fish (mullet *Mugil cephalus*), freshwater fish (crucian carp *Carassius auratus langsdorfii* and largemouth bass *Micropterus salmoides*), and aquatic bird (grey heron A*rdea cinerea*) were collected from Matsuyama, Ehime, Japan in 2005. Whole body homogenates (pooled from 3 individuals) of fish and muscle of bird sample were stored at –20 °C until analysis.

Analysis About 5 g of sample was homogenized in acetonitrile. ^{13}C-TCS and ^{13}C-MeO-TCS were spiked as surrogate standards and extracted twice with acetonitrile. The separation of phenolic compounds and its methoxylated analogues was carried out by hexane washing under basic condition as described by Okumura et al [14]. The acetonitrile extract was firstly partitioned by washing with hexane. The acetonitrile phase was added 5% NaCl solution and adjusted to pH 13 with NaOH, followed by washed twice with hexane under basic condition. After adjusting to pH 2-3 with HCl, the aqueous phase containing phenolic compounds was extracted with hexane twice. The combined extract was loaded on the column packed with deactivated silica gel column (2 g; 5% H_2O, w/w) and washed with hexane, followed by eluted with 30% dichloromethane/hexane. The eluent was concentrated and derivatized to the methoxylated analogues using trimethylsilyl-diazomethane which is more stable and less toxic than diazomethane, and than purified using sulfuric acid-silica gel column (1 g; 44% H_2SO_4, w/w). While, as the firstly partitioned hexane phase contains a small quantity of methoxy analogues originating in the samples, they were recovered by following step. The hexane phase was added acetonitrile saturated with hexane to remove lipids. The acetonitrile phase was collected and added 5% NaCl solution, and then extracted with hexane. Additionally, the hexane phase of washing under basic condition was combined. The combined hexane extract was passed through the sulfuric acid-silica gel column (2 g; 22% H_2SO_4, 2 g; 44% H_2SO_4, w/w) and eluted with 10% dichloromethane/hexane. Each purified extract was concentrated and spiked ^{13}C-PCB 79 as injection standard. Determination of the target compounds were carried out using HRGC (6890 series, Agilent technologies) / HRMS (JMS-800D, JEOL) at resolution of R>10,000 (10% valley). The gas chromatographic separation of each congener was performed using HT8-PCB column (60m × 0.25mm i.d, SGE Analytical Science). The recoveries of the target compounds in chicken muscle fortified with the native and ^{13}C-labelled standards at the concentration of 100 pg/g wet weight (ww) were in the range of 73-106% (n=3).

Results and Discussion

The results are shown in Figure 2. The concentrations of TCS in freshwater fish collected from the river in urban area were 3,370 pg/g ww for crucian carp and 3,730 pg/g ww for largemouth bass, which were two orders of magnitude higher than those of coastal fish (21 pg/g ww). It seems that these freshwater fish would be continuously exposed in contaminated river water. Levels of TCS in the whole body of largemouth bass analyzed in the present study was relatively similar to those in the plasma of same species from the Detroit River in North America, that concentration is 3,000 pg/g ww [8]. TCS concentration in aquatic bird (250 pg/g ww) was one order of magnitude lower than those of freshwater fish. While the reason for the observation is not clear, it is speculated that the less exposure or greater metabolism might be occurred in avian species.

The three chlorinated derivatives (TeCS Ⅰ, TeCS Ⅱ, PeCS) were observed in freshwater fish (36–150 pg/g ww) and bird (3.5–16 pg/g ww), but were consistently lower than TCS in each samples. These compounds were not detected in coastal fish. As we have also determined the TCS and its chlorinated derivatives in the river surface water from various locations in Japan, which data is presented by another short paper in this volume, the concentrations between fish and river water samples collected from the same location were compared. Although it should be noted that the sampling time of each sample is different, the concentration ratios of fish to river water were estimated at 2–3 orders of magnitude for these compounds, and indicated higher ratios for the

chlorinated derivatives than TCS. TeCS I were most abundant congener among the three chlorinated derivatives in freshwater fish, which were consistent with the results of river surface waters. On the other hand, more highly chlorinated congener PeCS was most abundant in bird sample. While there are few reports on the toxicity of the chlorinated derivatives, it has been observed that the acute toxicity and the effects on cytochrome P450 dependent monooxygenases in rodents were relatively higher than TCS [15].

MeO-TCS in freshwater fish samples were detected at 5,150 pg/g ww for crucian carp, 18,900 pg/g ww for largemouth bass, which were with in the range of other fish data previously reported [7, 9, 10]. MeO-TCS levels were consistently higher than TCS in fish samples as well as the river fish from German [7]. And our result was consistent with an earlier Japanese study [9], which indicating the tendency of high levels of MeO-TCS in freshwater fish compared to coastal fish. Although MeO-TCS in river water samples are not analyzed in our study, it has been found that the concentrations of MeO-TCS were consistently lower than those of TCS in river waters by other researchers [3, 4]. And it has been reported that the log P_{ow} for MeO-TCS and TCS are 5.00 and 4.35, respectively [16], suggesting that MeO-TCS is more lipophilic and bioaccumulative than TCS. However, MeO-TCS concentration in bird (44 pg/g ww) was lower than TCS in the same sample and MeO-TCS in freshwater fish. Because of the result from only one bird sample, it is difficult to elucidate the observation in the present study.

Interestingly, the methoxy analogues of the three chlorinated derivatives were detected in all samples, but were lower than MeO-TCS. These concentrations in freshwater fish ranged 91–230 pg/g ww for crucian carp and 260–710 pg/g ww for largemouth bass, which were the similar or higher compared to those of each hydroxy ones. It seems that these methoxy analogues possibly being formed from their hydroxy ones by microbial methylation in natural environment or wastewater treatment plant [3, 16]. In addition, the possibility of biomethylation of TCS in the liver or intestinal microflora in fish has been pointed out [8]. Koistinen et al. [17] reported that several homologues of polychlorinated phenoxyanisoles (PCPAs; polychlorinated methoxydiphenyl ethers, MeO-PCDEs) which are considered as chlorophenol impurities and biomethylation products were found in the sediment, mussels, and pike collected from the Finnish watercourses polluted by industry, and salmon, seal, and eagle from Baltic Sea. And in mussel incubation experiment, the tendency of higher bioaccumulation potential for MeO-PCDEs than for their hydroxy analogues has been observed [18].

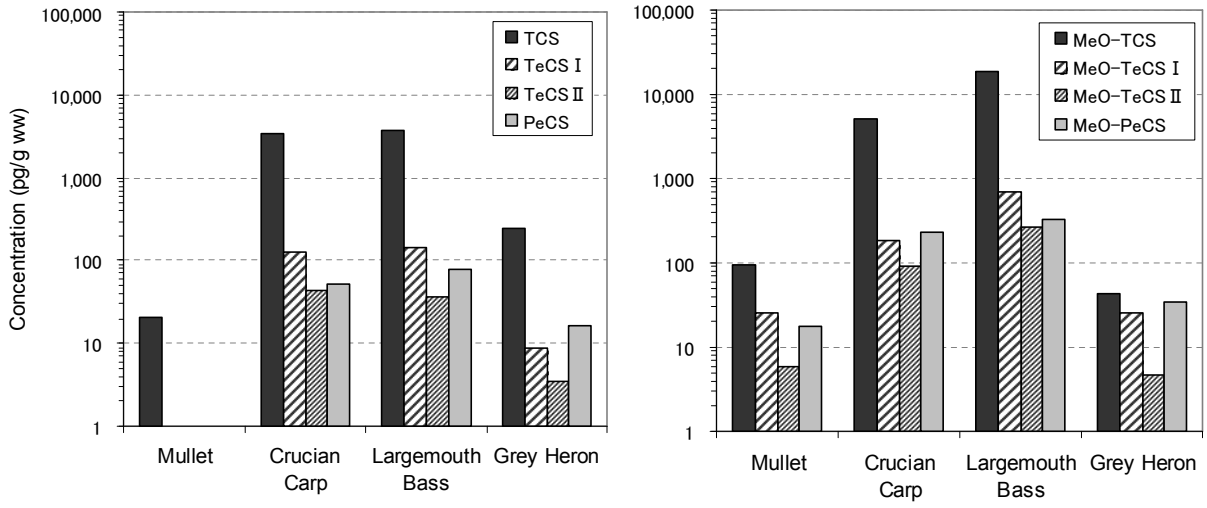

Fig. 2. Concentrations of TCS, its chlorinated derivatives, and their methoxylated analogues in mullet, crucian carp, largemouth bass, and grey heron.

Moreover, we examined whether the target analytes other than TCS are contained in TCS formulation as impurities. It was found that only minute amount of MeO-TCS was detected in Irgasan® DP300. MeO-TCS is an intermediate material in the production of TCS and thus a potential impurity has been suspected [3]. Whereas the other target analytes were not found. The result suggests that the chlorinated and their methoxylated derivatives of TCS might be unintentionally formed by chlorination reaction during the chlorine bleaching and sterilizing processes, and by biological methylation, respectively. To our knowledge, this is the first observation for the occurrence of the chlorinated derivatives of TCS and their methoxylated analogues in wildlife such as fish and bird, but quite limited number of samples. Further study on the environmental contamination and toxic effects of TCS and its related compounds will be necessary.

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