Determination of Toxaphene in Pintail Tissues from Lake Hyoko, Japan

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Introduction

An international convention aiming to restrict persistent organic pollutants (POPs) was formally adopted in May 2001; global actions to reduce and eliminate releases of the pollutants have been recommended. As we know, the compounds as the dirty dozen have already provided strong impact for wildlife and human beings. Although a large number of studies have been made on POP contamination in Japanese environments, little is known about toxaphene levels. Probably, the situation is derived from the fact that the compound has never been used in Japan. So far as atmospheric transport of POPs is concerned, toxaphene may also distribute in Japanese biota. In January 2003, we carried out a sampling survey of pintails at Lake Hyoko, Niigata, Japan. Concentrations of toxaphene were determined by gas chromatography-mass spectrometry (GC-MS) based on electron capture negative ionization (ECNI). The concentration levels among fat, breast muscles, and livers were discussed from the obtained data.

Material and methods

In January 2003, pintails were collected at Lake Hyoko in Niigata, Japan (Fig. 1). After determination of biological parameters, the samples were dissected, and were stored at –20 °C in sealed glass containers before analyses.

Toxaphene standard mixture (P11, P12, P15, P21, P25, P26, P31, P32, P38, P39, P40, P41, P42, P44, P50, P51, P56, P58, P59, P62, P63, and P69) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). $^{13}C_{10}$-*trans*-chlordane and $^{13}C_{12}$-PCB 153 were used as a surrogate standard and an internal standard, respectively. Both the labeled standards were purchased from Cambridge Isotope Laboratories (MA, USA). Solvents for dioxin analysis grade were employed (Wako Pure Chemical Industries, Osaka, Japan) and were used without further purification.

The analytical method is divided into an extraction step and a cleanup step. Pieces of fat tissue (1 g), breast muscles (5 g) and livers (5 g) were taken out from glass containers. A hydromatrix homogenate was transferred into a pressure-tight stainless steel column for accelerated solvent extraction system ASE-200 (Dionex, CA, USA), volume 33 ml. Extraction was carried out with acetone/hexane (1:1; v/v) at 100 °C within 10 min (5 min static and 5 min heating time under a pressure of 13.8 MPa). After the extraction, the ASE system was purged for 1 min. These

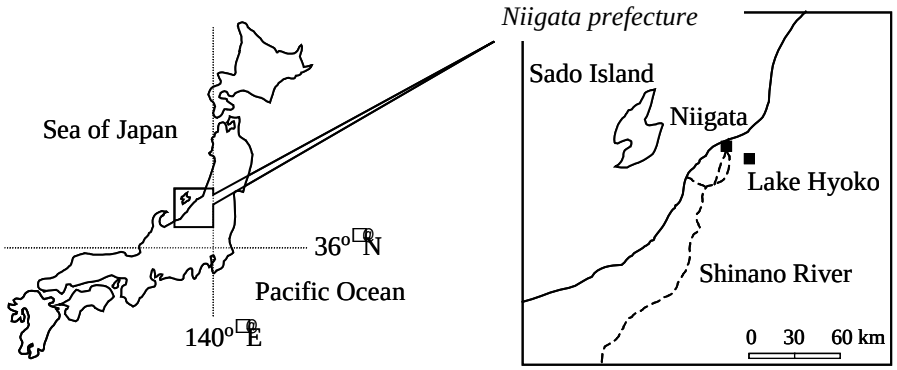

Figure 1: Map of Japan showing Lake Hyoko.

sampleswere extracted twice and all extracts were mixed (an extract and a purging solvent), and surrogate standards (10 ng) were added to mixtures. The volume was reduced in a rotary evaporator (BÜCHI, Flawil, Switzerland). Gel permeation chromatography (GPC) was performed to eliminate extra lipid of the ASE extracts. A GPC column (25 mm i.d., 500 mm length) was packed in our laboratory with 50 g of Bio-Beads® S-X3, 200–400 mesh (Bio-Rad, CA, USA) using mixture solution of dichloromethane/cyclohexane (1:1; v/v). A fraction between 125 ml and 275 ml was transferred to a rotary evaporator, and the fraction was concentrated to 5 ml. Hexane was added to replace dichloromethane/cyclohexane in solution during reduction of the sample volume. The concentrate was further purified with a glass column (15 mm i.d., 300 mm length) containing from top to bottom: 2 g of anhydrous sodium sulfate, 10 g of florisil (Wako Pure Chemical Industries, Osaka, Japan: activated 130 °C, 18 h), 2 g of anhydrous sodium sulfate and a quartz wool plug. The column was pre-rinsed with 50 ml of diethyl ether/hexane (5:95; v/v). The hexane concentrate was quantitatively transferred to the column and eluted with 100 ml of diethyl ether/hexane (5:95; v/v). This fraction was then evaporated to 5 ml for silica gel column chromatography. A glass column (12 mm i.d., 300 mm length) containing from top to bottom: 2 g of anhydrous sodium sulfate, 5 g of Silica gel 60 (Merck, Darmstadt, Germany: activated 130 °C, 18 h), 2 g of anhydrous sodium sulfate and a quartz wool plug. After conditioning the column with 50 ml of hexane, the concentrate was then eluted first with 30 ml of hexane. The fraction containing toxaphen was collected with 30 ml of diethyl ether/hexane (25:75; v/v) and was transferred to centrifuge tubes. The volume reduced with a gentle stream of dry nitrogen. Five hundred picograms of $^{13}C_{12}$-labeled PCB 153 as an internal standard was added to the final concentrate.

Determination of toxaphene was performed by a portable mass spectrometer 5973N Mass Selective Detector (Agilent Technologies, DE, USA) equipped with a 6890 series gas chromatograph

(Agilent Technologies, DE, USA). HT8 (SGE Japan, Kanagawa, Japan: 50 m length, 0.22 mm i.d., 0.25 μm film thickness) was selected as a fused silica capillary column. Helium was employed as a carrier gas at the flow rate of 1 ml min^{-1}. One microliter of a final concentrate was injected with an autosampler 7673 (Agilent Technologies, DE, USA) under a pulsed splitless mode. Temperatures of an injector port and a transfer line in gas chromatograph were maintained at 220 °C and at 280 °C, respectively. The column temperature was maintained at 60 °C for 1 min, ramped to 170 °C at a rate of 23 °C min^{-1}, 7.5 min isothermal, to 275 °C at a rate of 3 °C min^{-1}, and maintained at 275 °C for 12 min. Methane was employed as a reagent gas. Temperatures of an ion source and a quadrupole were held at 150 °C and 106 °C, respectively. The mass spectrometer was operated on the basis of selected ion monitoring (SIM).

Results and Discussion

The concentrations were presented as means based on a lipid weight basis (l.w.). Mean lipid contents were as follows: 81.9% (fat), 1.5% (muscles), and 5.5% (livers). Mean recoveries of $^{13}C_{10}$-*trans*-chlordane ranged from 87.1% to 88.4% with the relative standard deviations of 5.4% or less.For accurate determination of toxaphene, measurement by GC-ECNIMS was limited to the 22 congeners which could purchase commercially available standard; as a result, the profile was dominated by two congeners, P26 (octachlorobornane, B8-1413) and P50 (nonachlorobornane, B9-1679). Toxaphene congeners except P26 and P50 were absent in all the samples; therefore, thesum of P26 and P50 denoted the overall toxaphene concentration.

Table 1: Concentrations (ng g^{-1} l.w.) of toxaphene in fat, breast muscles, and livers.

Congener	fat, n = 11			breast muscles, n = 11			livers, n = 11		
	mean	min.	max.	mean	min.	max.	mean	min.	max.
P26	0.8	0.2	1.7	1.2	0.0	3.8	0.2	0.0	0.4
P50	0.5	0.1	1.2	0.8	0.0	3.3	0.0	0.0	0.1
Sum	1.2	0.3	2.9	1.5	0.0	6.0	0.3	0.0	0.5

Toxaphene, like DDT products, was distributed worldwide, and yet it was never registered as a pesticide in Japan. In particular, the compound was distributed at high concentrations in the North Atlantic countries and in eastern North America[1]. Table 1 indicated that the residue levels (0.3–2.9 ng g^{-1} l.w., fat; n.d.–6.0 ng g^{-1} l.w., muscles; n.d.–0.5 ng g^{-1} l.w., livers) were extremely low compared to those of the other OCs. Further, the mean concentrations (approximately 1 ng g^{-1}) were also considerably lower than those in penguin fat (P26, 34 ng g^{-1}; P50, 23 ng g^{-1})[2]. The minor nature of toxaphene's contribution to the overall OC contamination differed from substantial contribution extent of the data (albatross fat tissues from the north Pacific Ocean) reported by Muir et al[1]. As we know, however, avian species and sampling locations have been deemed the causes of the major differences in toxaphene contribution between the two sets of data. Only the two prominent congeners (P26 and P50) were found among 22 analyzed CHBs in our pintails. P50 was capable of being detected as several research groups have reported the selective bioaccumulation of the congener in nonachlorobornanes [3,4]. The concentration ranges of P50 were as follows: 0.1–1.2

ng g^{-1} l.w. (detected in all the fat tissues), n.d.–3.3 ng g^{-1} l.w. (detected in three muscle samples), and 0.1 ng g^{-1} l.w. (detected in one liver sample). On the other hand, P26 was the more dominant contributor than P50 and ranged from 0.2–1.7 ng g^{-1} l.w. (detected in all the fat tissues), from n.d.–3.8 ng g^{-1} l.w. (detected in nine muscle samples), and from n.d.–0.4 ng g^{-1} l.w. (detected in nine liver samples). In the present study, the concentration ratio ([P26]/[P50]) of the two congeners in fat tissues ranged from 1.2 to 2.9, and these residue patterns were the reverse of the patterns of cetaceans from the northern hemisphere. In contrast, the ratios ([P26]/[P50]) in albatrosses from the northern hemisphere approximated 1.0. Therefore, we concluded that these patterns originated from differences of the species (birds, fishes, marine mammals, and others) or dietary habits rather than differences in volatility among toxaphene congeners in the hemisphere.

To our knowledge, this is the first study to show residue levels of toxaphene in Japanese biota. Lipid in breast muscles was shown to be capable of being a final sink for migratory birds; we may, therefore, reasonably conclude that the distribution of toxaphene stems from the motion of breast muscles during migration. In addition to this, the assumption that toxaphene contamination had spread to the Far East, including Japan, has at least been validated.

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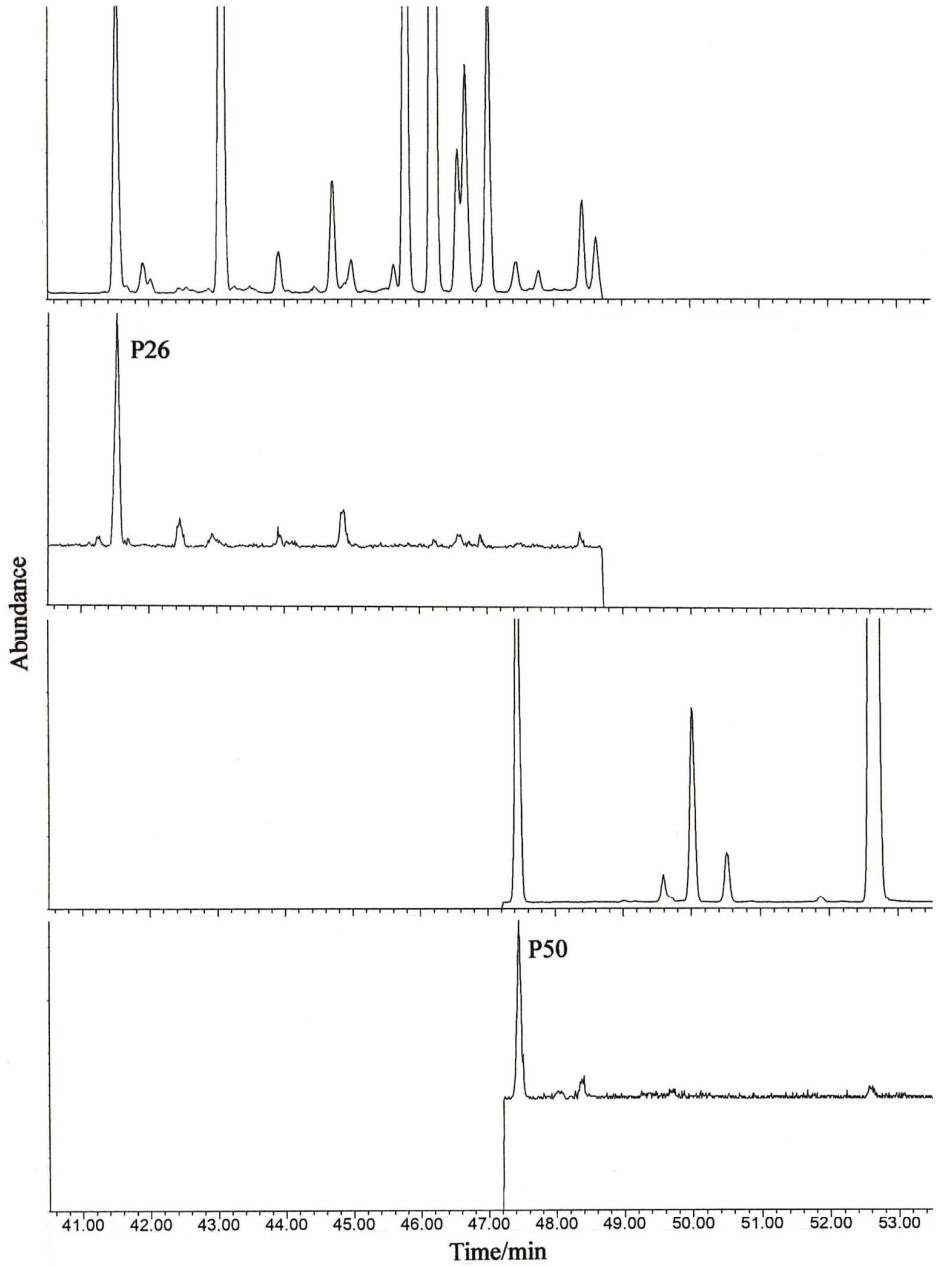

Figure 2: SIM chromatograms in the pintail fat tissue. top: STD (octachlorobornanes); second from the top: sample; second from the bottom: STD (nonachlorobornanes); bottom: sample

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