Ecotoxicology and Environmental Safety 147 (2018) 367–372

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Persistent organic pollutants in red-crowned cranes *(Grus japonensis)* from Hokkaido, Japan

Kensaku Kakimoto[a,*], Kazuhiko Akutsu[a], Haruna Nagayoshi[a], Yoshimasa Konishi[a], Keiji Kajimura[a], Naomi Tsukue[b], Tomoo Yoshino[c], Fumio Matsumoto[c], Takeshi Nakano[d], Ning Tang[b], Kazuichi Hayakawa[b], Akira Toriba[e]

[a] *Osaka Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan*
[b] *Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan*
[c] *Kushiro Zoo, Shimoninishibetsu-11 Akan-cho, Kushiro, Hokkaido 085-0201, Japan*
[d] *Research Center for Environmental Preservation, Osaka University, 2-4, Yamadaoka, Suita, Osaka, 565-0871, Japan*
[e] *Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan*

## ARTICLE INFO

*Keywords:*
Persistent organic pollutants
Red-crowned crane
Muscle tissue
*Grus japonensis*
Endangered birds

## ABSTRACT

The red-crowned crane (*Grus japonensis*) from eastern Hokkaido is classified as a Special Natural Monument in Japan. In this study, we determined the concentrations of persistent organic pollutants (POPs) in red-crowned crane muscle tissues (n = 47). Polychlorinated biphenyls (PCBs) had the highest median concentration (240 ng/g lipid weight), followed by dichlorodiphenyltrichloroethane and its metabolites (DDTs) (150 ng/g lipid weight), chlordane-related compounds (CHLs) (36 ng/g lipid weight), hexachlorobenzene (HCB) (16 ng/g lipid weight), hexachlorocyclohexanes (HCHs) (4.4 ng/g lipid weight), polybrominated diphenyl ethers (PBDEs) (1.8 ng/g lipid weight), and finally, Mirex (1.5 ng/g lipid weight). Additionally, a positive correlation was found among POP concentrations. No sex differences beyond body parameters were observed. Additionally, red-crowned cranes exhibited a high enantiomeric excess of (+)-*alpha*-HCH, with enantiomer fractions varying from 0.51 to 0.87 (average: 0.69).

## 1. Introduction

Endemic to eastern Hokkaido, Japan, the red-crowned crane (*Grus japonensis*; Fig. 1) is a designated Special Natural Monument of the nation. This non-migratory island population is one of two populations worldwide, according to the International Union for Conservation of Nature and Natural Resources (IUCN); the other is a migratory continental population in northeast Asia (BirdLife International, 2016). The Japan population faced an extinction crisis in the late 19th century due to hunting and habitat destruction stemming from extensive industrial and agricultural development (Masatomi et al., 2007). Subsequent conservation efforts included habitat protection (e.g., designating the Kushiro Marshland as a national park) and artificially supplementing the cranes' omnivorous diets with dent corn during winter. (Red-crowned crane diets consist of agricultural crops, nuts, insects, frogs, fish, shellfish, and crabs; Kobayashi et al., 2002.) The Hokkaido government's 2015 distributional survey indicated that approximately 1000 cranes inhabited eastern Hokkaido following the implementation of conservation activities (Hokkaido Government, 2015).

The red-crowned crane is now classified as "endangered" and "vulnerable" according to the IUCN Red List of Threatened Species and Japan's Ministry of the Environment, respectively. This status is of particular concern because the crane populations have low genetic diversity coupled with limited, making the species susceptible to infections and toxic chemical compounds (Hasegawa et al., 1999). Indeed, red-crowned cranes have died from high pesticide intake (Takazawa et al., 2004). Heavy metal contamination was also reported in red-crowned cranes (Teraoka et al., 2007a, 2007b).

Persistent organic pollutants (POPs) have endocrine-disrupting and other toxic effects on avian species. These include thinning eggshells (Elliott et al., 1988), as well as lowered hatching rates, chick survival (Bustnes et al., 2008), hatching sex ratio, body mass (Erikstad et al., 2011), egg weight/volume (Van den Steen et al., 2009), reproductive success (Marteinson et al., 2010), and thyroid hormone and vitamin A concentrations (Fernie et al., 2005). In Japan, POPs such as PCBs (polychlorinated biphenyls), DDT (dichlorodiphenyltorichloroethane), and HCHs (hexachlorocyclohexane) were in use from 1950s, and then banned during 1970s. Chlordane related compounds (CHLs) was used

* Corresponding author.
*E-mail address:* knkakimoto@iph.osaka.jp (K. Kakimoto).

http://dx.doi.org/10.1016/j.ecoenv.2017.08.060
Received 1 June 2017; Received in revised form 24 August 2017; Accepted 28 August 2017
Available online 14 September 2017

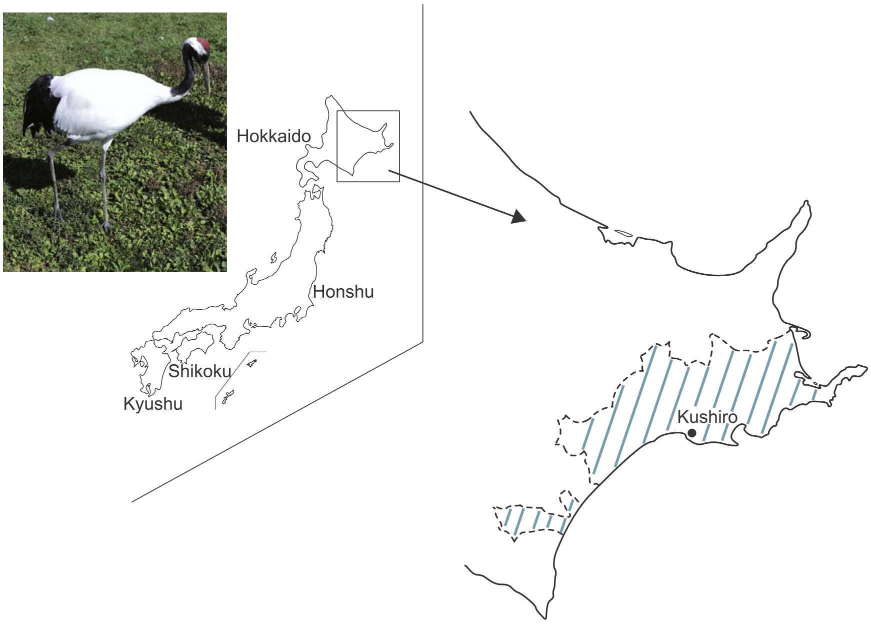


**Fig. 1.** Map of red-crowned crane distribution in eastern Hokkaido, Japan. The shadowed area indicates the region in which bird specimens were collected (after dying from accidents).

**Table 1**
Body parameters of red-crowned cranes (*Grus japonensis*) from Hokkaido, Japan[a].

| | median | mean ± S.D. | min. | max. |
|---|---|---|---|---|
| body weight (kg) | 7.6 | 7.7 ± 1.3 | 5.0 | 10 |
| whole length (mm) | 1300 | 1300 ± 89 | 1000 | 1400 |
| fat of thigh muscle (%) | 2.0 | 2.7 ± 2.0 | 0.6 | 10 |

[a] Cranes were collected between 2002 and 2016. (n = 47).

from 1950s and banned in middle 1980s, and polybrominated diphenyl ethers (PBDEs; tetra to hepta-BDE) was banned in 2010 in Japan. Unfortunately, all of these POPs remain detectable in Japan's natural environment, including in animals (Hoshi et al., 1998; Kumar et al., 2005; Kunisue et al., 2008; Anezaki and Nagahora, 2014).

Currently, red-crowned crane habitats in Japan overlap with human environments, due to the fact that the birds have begun to build nests at marsh edges. As marshlands in eastern Hokkaido become depleted, increased human contact elevates the risk of POP exposure. The bioaccumulative nature of these environmental contaminants and their adverse effects on wildlife are of great concern to conservationists. In this study, we therefore determined POP levels in red-crowned cranes of Hokkaido through sampling their thigh muscle tissues. We also conducted enantiomer selective analyses of *alpha*-HCH to understand how red-crowned cranes may metabolically process contaminants. These results will contribute to our knowledge regarding the effects of POPs on this culturally important species and its marshland habitat.

## 2. Materials and methods

### 2.1. Chemicals

Standard mixes of the following compounds were purchased from Cambridge Isotope Laboratories (MA, USA): non-labeled organochlorine pesticide mix (hexachlorobenzene [HCB], 2,4′-DDT, 4,4′-DDT, 2,4′-dichlorodiphenyldichloroethane [DDD], 4,4′-DDD, 2,4′-dichlorodiphenyldichloroethylene [DDE], 4,4′-DDE, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, oxychlordane, *alpha*-HCH, *beta*-HCH, *gamma*-HCH); $^{13}$C-labeled organochlorine pesticide mix (HCB, 2,4′-DDT, 4,4′-DDT, 4,4′-DDE, *trans*-nonachlor, oxychlordane, *beta*-HCH); non-labeled/labeled PCBs; and PBDEs. Pesticide analysis-grade organic solvents were used for the extraction and cleanup of samples. Dioxin analysis-grade sulfuric acid-impregnated silica gel was purchased from Wako Pure Chemicals (Osaka, Japan)

### 2.2. Sample collection and preparation

Red-crowned cranes died mainly following traffic accidents or collisions with power lines between 2002 and 2016 in the periphery of Kushiro city were stored at −20 °C in the Kushiro Zoo with permission from the Japanese Ministry of the Environment. Facing the Pacific Ocean, Kushiro City is situated in southeastern Hokkaido, the northernmost island of Japan (Fig. 1). The Kushiro Marshland lies on the north side of Kushiro city. Thigh muscle samples were collected from 47 of the stored cranes (25 males and 22 females). Table 1 provides body parameters of the red-crowned cranes examined in this study.

### 2.3. Chemical analysis

The extraction and cleanup procedures used to prepare the thigh muscle samples were conducted as previously described (Kakimoto et al., 2012). Briefly, 10 g samples were spiked with $^{13}$C-labeled standard mixtures in the following amounts: 2,4′-DDE and 4,4′-DDE, 2.5 ng each; tetra- and penta-BDE, 0.25 ng each; hexa-, hepta-, and octa-BDE, 0.5 ng each; other target compounds, 1 ng each. Samples were extracted twice using a mixture of n-hexane and diethyl ether (2:1, v/v) in a homogenizer. After centrifugation, the organic layer was collected, washed with 2% NaCl, and then dehydrated with $Na_2SO_4$. The organic solvent was removed through evaporation, and the lipid content per sample was calculated based on the ratio of lipid weight to thigh muscle weight. Lipids were re-dissolved in an acetone-cyclohexane mixture (3:7, v/v), then purified using gel permeation chromatography. The eluate was evaporated to dryness before being re-dissolved again in hexane. The resulting solution was added to a 44% sulfuric acid-impregnated silica gel column (0.5 g), with hexane (10 mL) as an eluent. After the addition of nonane (20 µL), the eluate was evaporated to completely remove hexane. Finally, 1 µL of the sample solution was used for gas chromatography-mass spectrometry (GC/MS).

In a preliminary study using two cranes, we confirmed that the concentrations of target compounds in thigh-muscle lipids were comparable to concentrations in other tissues lipids of the same crane, including the stomach, cervical, and breast regions. In this study, we report the POP concentrations on a lipid-normalized basis.

GC/MS analyses were performed using a gas chromatograph (6890