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Accumulation properties of inorganic mercury and organic mercury in the red-crowned crane *Grus japonensis* in east Hokkaido, Japan

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ABSTRACT

The red-crowned (Japanese) crane *Grus japonensis* is native to east Hokkaido, Japan, in contrast to the East Asia mainland. Previously, we reported that red-crowned cranes in Hokkaido were highly contaminated with mercury in the 1990s and that the contamination rapidly decreased to a moderate level in the 2000s. In the present study, we determined levels of organic mercury (O–Hg) in the liver and kidney of cranes in east Hokkaido in comparison with levels of total mercury (T–Hg). T–Hg levels in the kidneys were higher than those in the livers in adults but not in subadults and juveniles; however, the reverse was the case for O–Hg even for adults. The ratio of O–Hg to T–Hg in both the liver and kidney decreased as T–Hg increased in the three developmental stages. While the ratios of O–Hg to T–Hg in the liver and kidney of adults were significantly lower than those of juveniles, the ratios were similar for adults and juveniles in a lower range of T–Hg. The ratio of selenium (Se) to T–Hg decreased as T–Hg increased in both the liver and kidney, irrespective of stages. Mercury burdens in feathers were about 59% and 67% of the total body burdens for juveniles and adults, respectively. Furthermore, ratios of carbon and nitrogen stable isotopes to T–Hg varied greatly, with no relation to mercury level in the liver. The results suggest slow accumulation of inorganic mercury in the kidney of red-crowned cranes in east Hokkaido, Japan.

1. Introduction

The red-crowned (Japanese) crane *Grus japonensis* is an endangered bird that is found mainly in wetlands (Meine and Archibald, 1996; Masatomi, 2000). In contrast to mainland populations, which are migratory between the Amur River basin and east China-Korean Peninsula, the non-migratory population is native to the east area of Hokkaido in Japan (island population). Red-crowned cranes are omnivores and eat small fish, worms, insects, frogs, plants, and grain in and near the wetlands from spring to fall. In winter, they are fed with corn and fish in part at several feeding stations in east Hokkaido instead of migration to some warm land (Masatomi, 2000). In the winter of 2010, 1324 cranes were confirmed to be present in east Hokkaido-almost half of the world population of approximately 2800 cranes in the wild (Masatomi, 2009). They are designated as a Special National Natural Monument by the Japanese government and are also highly protected birds in neighboring countries. They have been listed by the International Union for Conservation of Nature and Natural Resources (IUCN).

Mercury is a persistent and bioaccumulative toxic metal in wild birds as well as other wild animals and humans. Mercury is taken up from their food mainly as methylmercury (MeHg) and is then transformed into a less toxic form, inorganic mercury (I–Hg), in the liver and other internal organs (Ikemoto et al., 2004). Mercury in internal organs is stored in the form of I–Hg, at high concentrations in bird species (Thompson and Furness, 1989). Selenium (Se) forms complexes with I–Hg at an equimolar ratio in higher-trophic marine animals (Koeman et al., 1973; Palmisano

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et al., 1995) and seabirds (Kim et al., 1996b). It is well known that mercury is preferentially deposited in feathers in the form of MeHg, and molting is the most important excretion route for most bird species (Lewis and Furness, 1991). Exceptionally, however, in black-footed albatross *Diomedea nigripes*, large amounts of mercury accumulate in the liver than in the kidney without apparent poisoning (Kim et al., 1996a, 1996b). One of the characteristic features of the black-footed albatross is that the mercury burden in feathers is less than 10% of the body burden, in contrast to about 60–70% in most other birds (DesGranges et al., 1998). Instead, mercury in the liver accounts for about 50% of the body burden of mercury and is present predominantly as I-Hg. As feathers reflect blood MeHg level during feather growth (Bearhop et al., 2000), these observations were explained by their specifically higher capability of accumulation and demethylation in the liver as the prominent resistance of this species to mercury, because neurotoxicity of methylmercury is much greater than that of inorganic mercury (Kim et al., 1996b).

We previously reported that T–Hg levels in the livers and kidneys from red-crowned cranes that were found dead in east Hokkaido in the 1990s were very high (Teraoka et al., 2007). Since about 2000, a rapid decline in Hg levels has been recognized on the basis of average values, though some cranes still showed high mercury concentrations (Teraoka et al., 2012). This indicates that the source of mercury contamination existed somewhere in their habitat before the 1990s and was mostly eliminated thereafter. However, the possibility of a specific mercury accumulation property in red-crowned cranes cannot be ruled out, since there has been no evidence of mercury contamination in east Hokkaido other than that provided by us for cranes (Teraoka et al., 2007, 2012).

In the present study, levels and distribution of the burdens of T–Hg and O–Hg in the liver, kidney, feathers and some other tissues in red-crowned cranes in east Hokkaido were determined to obtain an insight into the mercury metabolism in this species and the contamination source of mercury. Additionally, a stable carbon isotope ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) were also analyzed to search for the possible cause of the higher mercury level, since these analyses have been used to address the trophic and dietary status of some bird species (Thompson and Furness, 1995; Bryan et al., 2012).

2. Materials and methods

2.1. Samples

Livers, kidneys, muscles and other tissues were obtained from red-crowned cranes *G. japonensis*, found dead or moribund in the wild, from January 1990 to February 2009 (Teraoka et al., 2012). We excluded cranes that were kept alive for more than 30 days and died in Kushiro zoo and other facilities. Developmental stage was determined by feather pattern and features on the forehead and crown, as previously described (Inoue et al., 2013). Adults (two years old or older) were mostly covered with white feathers, except for the black neck, secondaries and tertials. The crown was bright red without feathers. Subadults were covered with white feathers for most body parts and were very similar to adult birds except for black spots on the tips of the primaries and primary coverts and except for individuals in which crowns were not completely exposed. Juveniles, which were able to fly as distinguished from late chicks, were a combination of white, partly tawny, cinnamon brown and grayish.

Approximately 1 to 2 g samples of livers, kidneys and muscles were collected after removing the surface layer with a stainless steel knife (Feather, Osaka, Japan) and were dried for 12 h at 80 °C, as described previously (Teraoka et al., 2007). Skins and scales, collected with a stainless knife and feathers were washed in 3% Briji 35 by ultrasonication for 40 min, rinsed twice with acetone and ultrapure water, respectively, and then dried for 3 h at 80 °C. Dried samples were finely cut with a blender and kept in polyethylene bags (Teraoka et al., 2012). Polyethylene gloves were used throughout all dissection procedures to prevent contamination.

2.2. Analysis of total mercury

Total mercury (T–Hg) was determined with heat-vapor atomic absorption spectrometry (MA-2000; Nippon Instruments, Osaka, Japan), according to the company's instructions (Teraoka et al., 2012). In brief, cut samples of 200 to 500 mg (dry wt) were thermally decomposed at 800 °C and total mercury content was determined at 253.7 nm. All specimens were measured in batches that included blanks and a mercury standard (Kanto Chemical, Tokyo, Japan). Diluted mercury standard solutions (1.25, 2.5, 5, 10 ng) were used to make a standard line and obtained very good correlation ($R^2=0.99$). The Hg recoveries were $101.4 \pm 2.4\%$ ($n=5$) for the laboratory standards (human hair, National Institute for Environmental Studies [NIES] No. 13, NIES, Tsukuba, Japan), and the detection limit was 0.5 ng/g (dry wt). Standard mercury solutions for a standard line were measured before and after series of samples for each determination for calibration. Mercury concentration (T–Hg) of the organs was expressed as μg/g (dry wt).

2.3. Analysis of organic mercury

Organic mercury (O–Hg) was determined for a part of all livers and kidneys used for other analyses in this study. Organic mercury was extracted from tissues according to procedures reported by Miyamoto et al. (2010) with some modifications. Finely cut samples (500 mg, dry wt) were digested in a mixture of 0.1% cystein (1 mL) and 5 M sodium hydroxide (NaOH) (1 mL) at 70 °C for about 2 h. Ultrapure water (5 mL) and chloroform (5 mL) were added to the digests, followed by vigorous shaking (200 rpm, 10 min) and centrifugation (2000 rpm, 10 min). Hexane (3.6 mL) was added to the separated upper layer (5 mL) and vigorously shaken again. After centrifugation, 5 M hydrogen bromide (HBr, 1.5 mL), 1 M copper (II) chloride ($CuCl_2$, 0.5 mL) and toluene (6 mL) were added to the separated lower phase (2 mL). The separated upper layer (4 mL) was vigorously shaken with 0.1% cystein/1% acetate (1 mL). After centrifugation, the upper layer (toluene) was removed and the remaining toluene was evaporated from the lower layer by incubation at 70 °C. Mercury in the the remaining extracts in lower layer was determined with heat-vapor atomic absorption spectrometry (MA-2000), as mentioned above. Standard mercury solutions for a standard line were measured before and after series of samples for each determination for calibration.

Percent recoveries of MeHg were $102.6 \pm 3.2\%$ ($n=5$), $101.3 \pm 0.4\%$ ($n=5$) and $96.5 \pm 23.7\%$ ($n=5$) as estimated with standard materials (human hair, NIES No. 13; human hair, IAEA-085, International Atomic Energy Agency, Vienna, Austria; tuna muscle, CRMs-463, Institute for Reference Materials and Measurements, Geel, Belgium). Corrections were not carried out for each measurement, as the average of these recoveries was almost 100%.

2.4. Analysis of selenium

Concentrations of selenium (Se) were measured using an inductively coupled plasma-mass spectrometer (ICP-MS) (PMS-2000, Yokogawa Analytical Instruments, Tokyo, Japan) according to a previous study (Teraoka et al., 2007). Finely cut samples were digested in nitric acid and H_2O_2 using microwave digestion (MLS-