FLUORINATED SURFACTANTS AND OTHER PERESISTENT ORGANIC POLLUTANTS IN AN ENDANGERED WILD BIRD SPECIES, OKINAWA RAIL (*Gallirallus okinawae*)

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Abstract

Some of Persistent Organic Pollutants (POPs) and new POPs chemicals, i.e., organochlorine pesticides and fluorinated surfactants (PFCs), accumulated in tissues of an endangered bird species, Okinawa rail (*Gallirallus okinawae*) were analyzed. Although the levels of POPs pesticides in pectoral muscles were generally low, elevated levels of chlordanes and DDTs were detected in the birds found nearby villages along the coastline. On the other hand, some of PFCs, especially perfluoroalkyl carboxylic acids with odd number of carbon (perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFuDA)), were found in a few tens to a hundred ng g^{-1} level in more than half of the liver samples.

Introduction

Okinawa is a slender island located in sub-tropical ocean in a south western part of Japan. Major industries include agriculture (sugar cane and tropical fruits production) and sightseeing. Most of its 1.38 million population lives in south-western part of the island where military bases also locate. North eastern part, on the other hand, is a mountainous area, called YAMBARU in Japanese, which is covered with sub-tropical forests. Only a small number of people, about five thousands, live in several small villages along the coastline of YAMBARU, the deep forest of which still keeps natural habitats for many wildlife species.

In 1981, a new species of bird, Okinawa Rail, *G. okinawae*, was identified in YAMBARU (named YAMBARU KUINA in Japanese)[1]. Like Kiwi in New Zealand, it loses its ability to fly, and walks around forest floor to find its food, such as snails, insects, frogs, earthworm and other small animals[2]. Its habitat is limited in YAMBARU area with estimated total number of around 700, and it is nominated as natural monument and assigned as endangered species in category IA in Red Data Book compiled by the Ministry of the Environment, Japan. Since its discovery, much effort has been conducted to protect the species and their habitat, including control of its predators, construction of caged sanctuary and breeding facility. However, both their habitat area and the estimated total numbers have been gradually decreasing in recent years.

Its life is threatened by several factors; i.e., killed by small Asian mongoose (introduced in Okinawa in order to control a poisonous snake) or cats, loss of habitat by the development, and road-kill. As part of the scientific activities to protect

the species, cells and tissues of accidentally killed individuals have been collected and kept in Time Capsule facility at the National Institute for Environmental Studies. Here we report the chemical analysis data of tissues of accidentally killed Okinawa rail in order to understand their life and habitat better, to assess the quality of their environment, and to search for potential risks other than previously recognized ones.

Materials and Methods

Dead bodies of Okinawa rail, mostly by road-kill, once detected, were sent to our institute on ice. After rescuing live cells for cell / gene banking of endangered species, several tissues of the bird, such as pectoral muscle, liver and other organs (depending on the conditions of the specimen), were dissected out and were kept at -80 C.

For POPs analysis, typically c.a. 1 g of the pectoral muscle was weighed precisely, and was extracted with acetone/hexane by pressurized solvent extraction system (ASE-200, Dionex). The extracts, added with known amounts of stable-isotope labeled surrogates of organochlorine pesticides, were passed through a gel permeation column (BioBeads S-X3, BioRad Lab) to remove lipids and were clean-upped further with florisil and silicagel column chromatographies. Eight POPs pesticides (chlordane, DDTs, heptachlors, aldrin, dieldrin, endrin, HCB, mirex) were analyzed by GCMS (Agilent 6890 + 5973; NCI mode)[3]. For fluorinated surfactants analysis, 0.06 ~ 0.24 g of the liver was weighed and dissolved at 90 C for 3 hours with 2 ml of 4N NaOH and known amount of stable-isotope labeled surrogates. The solution was neutralized and mixed with an ion pair reagent, TBA, and extracted with MTBE (methyl tertiary-butyl ether). The extract was evaporated, dissolved in hexane, and was clean-upped with ChemElut cartridge column and acetonitrile, and analyzed by LCMSMS (4000 QTRAP, Applied Biosystems).[4]

Results and Discussion

It was reported that 23 individuals of Okinawa rail were killed in 2007 by traffic accidents among only 700 estimated individuals living in YAMBARU[2]. It is reported that each of adult Okinawa rail has rather narrow territory with several hundreds m in diameter. Thus the individual killed by the accident seems to have its own territory in that place and therefore represent the environment surrounding the location of the accident. In fact, the results of the POPs pesticides analysis in Okinawa rail could be interpreted to show the effect of local human activities consistently.

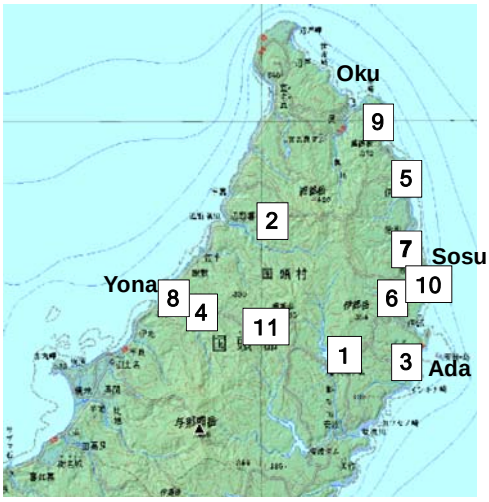

Figure 1 Sampling locations of Okinawa rail

Figure 1 shows the sampling locations of Okinawa rail analyzed of their POPs and PFCs levels in pectoral muscle and liver, respectively. The YAMBARU area is characterized by mountains covered with deep sub-tropical forests, which has been nursing variety of rare and threatened organisms, such as Okinawa rail, Okinawa woodpecker (*Saphaeopipo noguchii*), and Yanbaru long-armed scarab beetle (*Cheirotonus jambar*). There

are several small villages with small agricultural activities in the area along the coastline, including Ada, Yona, Sosu and

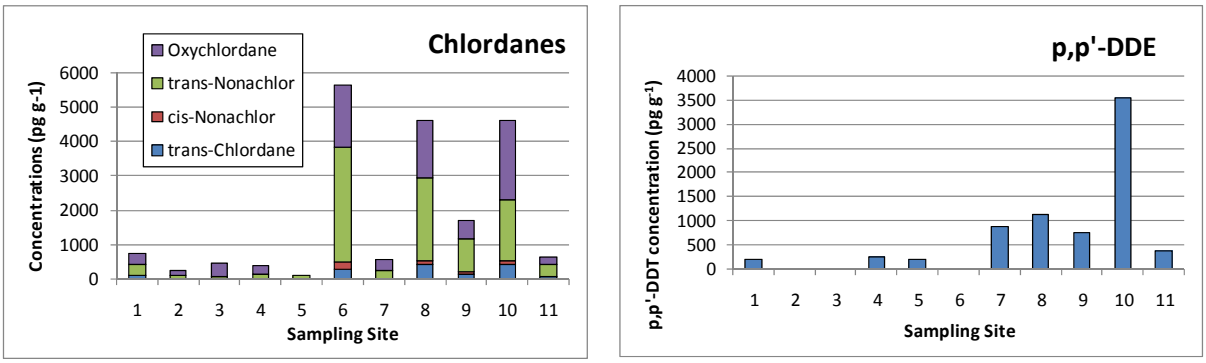

Figure 2 Chlordane (left) and *p,p'*-DDE (right) concentrations in pectoral muscle of Okinawa rail
See Figure 1 for sampling locations

Oku, which are shown in the figure 1.

Among POPs pesticides, chlordanes and *p,p'*-DDE levels are higher than others and their data are summarized in Figure 2. Relatively higher levels of chlordanes and *p,p'*-DDE could be detected in the tissues of Okinawa rail found near the villages, such as No. 6, No. 8 and No. 10, suggesting the historical usage of these chemicals nearby the residential areas. Under sub-tropical climate, termite control has been very important in houses in Okinawa Island, and chlordane had been used for the purpose. The data in Figure 2 seems to be consistent with the estimated historical usage of chlordane.

The levels of chlordanes and DDE in Okinawa rail are generally low compared with other reports in Japan. For example, Ministry of the Environment has been conducting environmental monitoring of POPs in wildlife from 1978, and conducted monitoring of Endocrine Disruptive Chemicals (EDCs) in wildlife from 1998 until 2006. Based on the recent report[5], total chlordane concentrations in pectoral muscle were reported to be 1500 ~ 2700 pg g^{-1} in juvenile black tailed seagull, 960 ~ 1200 pg g^{-1} in grey starling, 3400 ~ 14000 pg g^{-1} in cormorant, 1700 ~ 49000 pg g^{-1} in crow, respectively, and *p,p'*-DDE concentrations were 4700 ~ 20000 pg g^{-1} in juvenile black-tailed seagull, 94000 ~ 160000 pg g^{-1} in grey starling, 76000 ~ 370000 pg g^{-1} in cormorant, and 1800 ~ 97000 pg g^{-1} in crow, respectively. The highest chlordanes data of Okinawa rail are around 5000 pg g^{-1}, within distribution ranges of cormorant or crow, but other data are lower than any of the above reported values. *p,p'*-DDE levels of Okinawa rail were also lower than the values in other species. These data support the view that YAMBARU area, the habitat of Okinawa rail, is not contaminated with POPs pesticides except for local environments surrounding the residential areas.

On the other hand, fluorinated surfactants in the livers of Okinawa rail showed different distribution patterns (Figure 3). Perfluoroalkyl carboxylic acids (PFOA ~

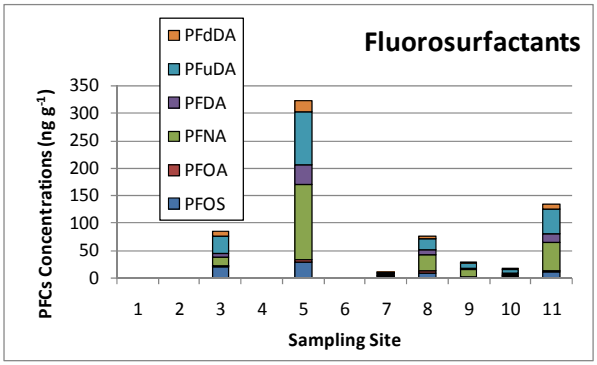

Figure 3 PFCs levels in livers of Okinawa rail

PFdDA) are the dominant PFCs in the livers with clear odd (PFNA, PFuDA) > even (PFOA, PFDA, PFdDA) preference characteristics. In many places along the road in YAMBARU, the sum of PFCs analyzed in the present study exceeded 50 ng g^{-1} wet weight in the livers, and their geological distribution patterns did not show clear relationship with population distribution in the area. There are many small tributaries in the YAMBARU area, but again no clear relationship could be pointed out between PFCs distribution and water flow system in the area. The levels of PFOS and PFOA are generally low, in the range of a few ng g^{-1} in wet basis; these are much lower values compared with the reported values of PFOS and PFOA in the livers of Japanese birds, including gull (<19 ~ 230 ng g^{-1}), duck (160 ng g^{-1}), heron (49 ~ 52 ng g^{-1}), kite (180, 450 ng g^{-1}) and cormorant (170 ~ 650 ng g^{-1})[6]. Sum of fluorinated surfactants analyzed in the present study, however, exceeds 50 ng g^{-1} in more than half of the locations with PFNA and PFuDA as dominant PFCs. In samples with elevated levels of PFCs, concentrations of PFNA and PFuDA are comparable to the PFOS levels reported in the literature[6].

It is not clear at this stage why perfluoroalkyl carboxylic acids are present in such concentrations in Okinawa rail. Characteristic pattern of perfluoroalkyl carboxylic acids with preference of compounds having odd-number of carbon atoms have been observed frequently in bivalves along coastline of Japan[4]. In our data[4], however, bivalves collected in Okinawa did not show elevated levels of PFCs compared with other areas, supporting the view that there is no strong industrial sources of PFCs in the island. As reported separately, we also found some locations in other area in Japan where elevated levels of PFCs were accumulated in insects[7]. These locations are mountainous areas like YAMBARU where no particular industrial activities, other than agriculture and forestry, are present. More research will be needed to clarify the reason of the presence of elevated levels of PFCs in Okinawa rail and their major sources in YAMBARU area.

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