Japanese Journal of Veterinary Research 62(3): 107-115, 2014

FULL PAPER

Contamination status and possibility of toxic effects of co-planar polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and dichlorodiphenyltrichloroethane in large Japanese field mouse (*Apodemus speciosus*) collected from Hokkaido and Aomori

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Received for publication, March 13, 2014; accepted, May 20, 2014

Abstract

Contamination levels of coplanar polychlorinated biphenyls (Co-PCBs), polycyclic aromatic hydrocarbons (PAHs), and dichlorodiphenyltrichloroethane (DDTs) were measured in the entire body of the large Japanese field mouse (*Apodemus speciosus*) collected from Hokkaido (Ishikari and Rankoshi) and Aomori prefecture (Takko) in Japan. Higher concentrations of PCBs including Co-PCBs, were observed in the mice collected from Ishikari than those from Rankoshi. The concentration of PAHs in the soil from Ishikari was also higher than that in the other sampling sites. The findings suggest that Ishikari is the most polluted area, probably because of human activities, depending on the population distribution. However, the observed contaminant levels were extremely lower compared to those in previous studies. The ratio of testis weight to body weight (TW/BW) was the lowest in the mice collected from Ishikari, which is the area contaminated with PAHs and *p,p*'-dichlorodiphenyldichloroethylene (DDE). However, the serum testosterone levels of mice from the Ishikari area were higher than those from the non-contaminated other areas although no significant differences. Previous studies have shown that a low-level exposure to dioxin related compounds (DRCs) disturbances in sexual function, resulting in the

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doi: 10.14943/jjvr.62.3.107

production of testosterone. This study showed that POPs exposure is one of the possibility of the high testosterone concentration in the mice of the Ishikari area in addition to a cause of biological and environmental factors such as habitat density, age, temperatures and/or food riches.

Key Words: Coplanar PCBs (Co-PCBs), Large Japanese field mouse, PAHs, Testis, Testosterone

Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), and polycyclic aromatic hydrocarbons (PAHs) are a group of pollutants that has sparked global health concerns. POPs have gained worldwide attention because of their ability to undergo bioaccumulation, high toxicity, and ubiquitous exposure to humans and wildlife. Most importantly, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar PCBs (Co-PCBs) are generally known as dioxin related compounds (DRCs), and these organochlorine contaminants are biomagnified as they pass through various trophic levels of the food web[15]. DRCs cause various toxic effects, including immune suppression, tumor promotion, and endocrine disruption such as impairment of reproduction after these enter the body through ingestion[21,22]. Endocrine disruption adversely affects reproduction in a wide range of species; these effects include a reduction in sperm production, reproductive failure, reproductive impairment, testicular atrophy, and decreased fecundity[4,18]. The effects of dioxin pollution on reproduction has been implicated in reports of reductions in the population size of the common cormorant (*Phalacrocorax carbo*) in Japan[19,20] and the American mink (*Mustela vison*) in the Great Lakes[27]. Although numerous studies have shown high levels of accumulated POPs in the Japanese wildlife, most have focused on aquatic animals[7,11,17] whereas only a few studies have examined the dioxin levels in terrestrial animals[13,24].

The large Japanese field mouse (*Apodemus speciosus*) is endemic to Japan. A previous study showed that certain environmental pollutants such as DDTs, PCBs, PCDDs, and PCDFs, accumulated in *Apodemus* species in the UK[16]. The large Japanese field mouse accumulates dioxins in its liver[9,25]. On the basis of facts from the literature, the species, therefore, is suitable for use as a bioindicator of the effects of exposure to dioxins. Certain wild rodents such as the large Japanese field mouse showed seasonal variations in the reproduction and behavior, which are regulated by the hypothalamus-pituitary-testis, or ovary axis[14]. During the breeding season, the testes of the rodent enlarged and the ratio of testis weight (TW) to body weight (BW) (TW/BW) became approximately twice more than that during the non-breeding season[14].

The present study examined the accumulation levels of PCBs, DDTs, Co-PCBs, and PAHs in the soil and in the large Japanese field mice collected from Hokkaido and Aomori prefecture, Japan. Furthermore, this study investigated the effects of the contaminants on the reproductive systems of the large Japanese field mouse.

Material and methods

Sample collections: All experiments using animals were performed according to the guidelines established by the University's Institutional Animal Care Use Committee. *A. speciosus* undergoes seasonal cycles of reproductive periods throughout the year, from April to October[14]. In this study, large Japanese field mice were collected from Hokkaido and Aomori prefecture

Table 1. Characteristics of the large Japanese field mice and soil samples included in this study

	Ishikari	Rankoshi	Takko
Area	Suburban and agricultural	Aguricultural	Forest
Sampling date	October 17, 2003	November 6–8, 2003	October 31 to November 3, 2003
Samples	Whole body (pool) Soil (n = 1)	Whole body (pool) Soil (n = 1)	Whole body (pool) Soil (n = 1)
Sex	Male	Male	Male
Body weight ± standard deviation (g)	29 ± 4.3 (n = 5)	33 ± 6.9 (n = 5)	27 ± 3.6 (n = 9)

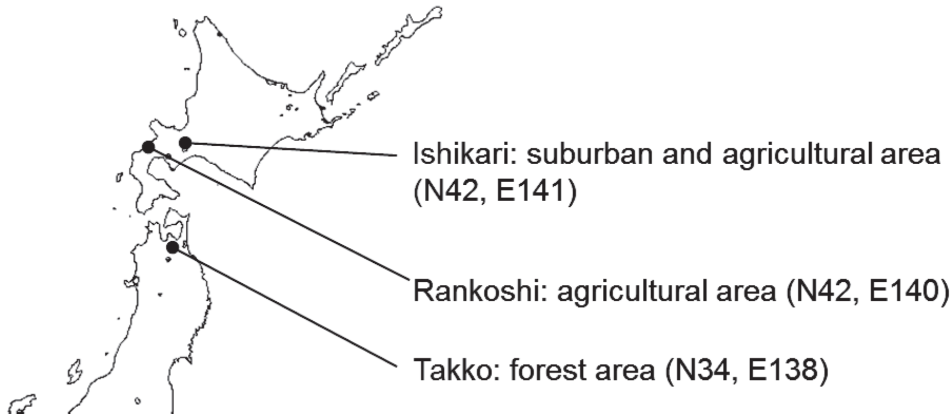

Fig. 1. Map showing the locations of the sampling points: Ishikari and Rankoshi in Hokkaido and Takko in Aomori prefecture.

in Japan during the end of the breeding seasons (October and November) in 2003 (Table 1, Fig. 1). Three sites were chosen as sampling areas in this study: a suburban and agricultural area (Ishikari: N42, E141) and an agricultural area (Rankoshi: N42, E140) in Hokkaido, and a forest area as a non-contaminated site (Takko: N34, E138) in Aomori prefecture. Adult male large Japanese field mice were caught using Sherman traps with oatmeal and sweet potatoes as baits. The traps were set in the evening and the entrapped mice were captured alive in the next morning. The large Japanese field mouse (*A. speciosus*) was distinguished from other wild rodent species such as the small Japanese field mouse (*A. argenteus*) by the difference in tail length, color, and hind foot length. Individuals with BWs > 25 g were classified as mature[1,23), and thus, were used for reproductive analyses. Five, five and nine male mice were collected from Ishikari, Rankoshi and Takko, respectively.

After collection, the mice were immediately sacrificed by using CO_2 gas and the following tissues were collected: hindbrain, liver, testis, blood, and adrenal gland. Right testis of each male mouse was weighed and the TW/BW was calculated. The blood samples were also collected from mice for measurement of serum testosterone level. All tissues and serum samples were frozen in liquid nitrogen and kept at −80°C until chemical analyses. Soil samples were also collected from each sampling area (Ishikari, n = 1; Rankoshi, n = 1; and Takko, n = 1).

Chemical analyses: The entire body of the large Japanese field mice and soil samples were used for congener analyses. Five to six bodies without hindbrain, liver, testis, blood, and adrenal gland were randomly selected from each mouse group, then, these bodies were pooled following numbers for chemical analysis; Ishikari (n = 1), Rankoshi (n = 1), and Takko (not analyzed). PAHs, DDTs, PCBs, and Co-PCBs were analyzed by Maxxam Analytics Inc. (Ontario, Canada) by using a gas chromatography (GC) system equipped with an electron capture detector (ECD) for DDTs and PCBs, low-resolution mass spectrometer (LRMS) for PAHs, and high-resolution MS (HRMS) for Co-PCBs. The analytical method was based on EPA Method 8081 for DDTs, 8270 for PAHs and 8290B for Co-PCBs and non-dioxin-like PCBs. Briefly, soil and biological samples were prepared by Soxhlet extraction. The extracts were subjected to an acid-base washing treatment and dried. Following a solvent exchange step, the extracts were purified by column chromatography on alumina, silica gel, and/or activated carbon, depending on the matrix. The preparation of the final extract for instrumental analysis was accomplished by adding a solvent solution containing the recovery standards, then the samples were injected into the instruments.

Toxicity equivalence quantity (TEQ) was calculated by using a set of toxic equivalence factors (TEFs) for fish, as proposed by the World Health Organization (WHO)[26].

Determination of serum testosterone concentration: The concentrations of serum testosterone were examined by using an enzyme immunoassay (EIA) method (Cayman Chemical Company, MI, USA), following the manufacturer's protocol. Serum samples were extracted using diethyl ether, and after vortex mixing, diethyl ether layers were transferred onto new glass tubes and dried by using N_2 gas. The testosterone extracts were dissolved in EIA buffer, and the hormone levels were measured based on color reaction.

Statistical analyses: The Tukey-Kramer test was used to identify significant differences in the concentrations of target compounds among the study areas. P-values less than 0.05 were considered significant.

Results

Mean BWs ± standard deviations of the male large Japanese field mice were measured (Table 1), and no significant differences were observed in the BW of male large Japanese field mice. On the other hand, significant differences in TWs were detected in the mature large Japanese field mice. The TW/BW values of the mice collected from Ishikari were the smallest among the sampling areas (Fig. 2).

The concentrations of the contaminants in the soil and whole body samples are presented in Table 2. Only PAHs (i.e. phenanthrene, fluoracene, pyrene, benzo[a]pyrene and benzo[b]fluoranthene) and *p,p*'-dichlorodiphenyldichloroethylene (DDE)

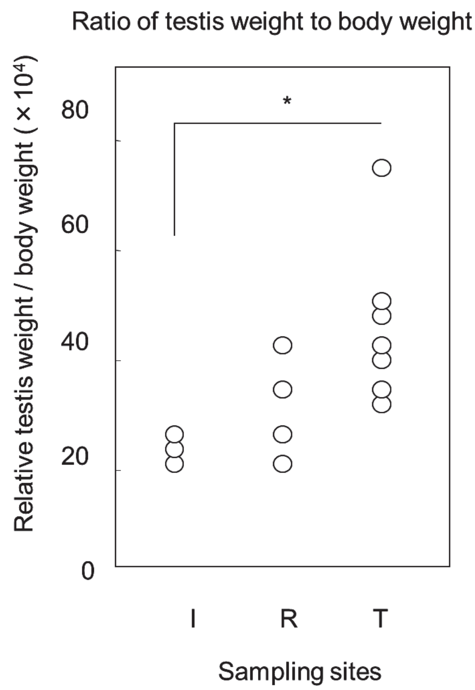

Fig. 2. Ratio of testis weight (TW) to body weight (BW) in large Japanese field mice. In the X-axis, I denotes Ishikari (n = 3) and R, Rankoshi (n = 4) in Hokkaido, and T is Takko (n = 7) in Aomori prefecture. One to two individuals in each area were excluded because testis weight was not available. *: $p < 0.05$

Table 2. Concentration of PAHs, DDTs (ng/g), non-dioxin like PCBs[a)] and Co-PCBs (pg/g) in the soil (wet-weight basis) (2-1) and in the pooled whole body of large Japanese field mice (lipid-weight basis) (2-2) collected from Ishikari and Rankoshi, Hokkaido, Japan

Table 2-1

	Soil		
Sampling area	Ishikari	Rankoshi	Takko
PAHs[b)]			
Phenanthrene	10	< 5.0	< 5.0
Fluorancene	21	< 5.0	< 5.0
Pyrene	17	< 5.0	< 5.0
Benzo[a]pyrene	16	< 10	< 10
Benzo[b]fluoranthene	13	< 5.0	< 5.0
DDTs			
p,p'-DDD	< 2.0	< 2.0	< 2.0
p,p'-DDE	6.0	< 2.0	< 2.0
p,p'-DDT	< 2.0	< 2.0	< 2.0

Table 2-2

Sampling area	Ishikari	Rankoshi
Lipid content (%)	2.3	2.9
PCBs		
Monochlorobiphenyl	< 35	< 35
Dichlorobiphenyl	< 900	< 900
Trichlorobiphenyl	< 300	< 300
Tetrachlorobiphenyl	4000	1800
Pentachlorobiphenyl	6500	< 64
Hexachlorobiphenyl	5700	1600
Heptachlorobiphenyl	2000	760
Octachlorobiphenyl	< 5.0	200
Nonachlorobiphenyl	< 5.0	< 5.0
Decachlorobiphenyl	< 5.0	< 5.0
Total PCBs	18000	4400
Co-PCBs[c)]		
3,3',4,4',5-PeCB (126)	52	< 0.70
2,3,3',4,4',5-HxCB (156)	570	< 8.0
2,2',3,3',4,4',5-HpCB (170)	480	< 10
2,2',3,4,4',5,5'-HpCB (180)	700	310
2,3,3',4,4',5,5'-HpCB (189)	< 3.0	110
Total Co-PCBs	1800	420
TEQ (pg-TEQ/g)	6.8	1.8

a): PCB levels of the soil samples were below the detection limit.
b): The levels of naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, benz[a]anthracene, chrysene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[ghi]perylene in all samples were below the detection limit.
c): The levels of 3,3',4,4'-TeCB (77), 2,3,3',4,4'-PeCB (105), 2,3,4,4',5-PeCB (114), 2,3',4,4',5-PeCB (118), 2',3,4,4',5-PeCB (123), 2,3,3',4,4',5'-HxCB (157) and 3,3',4,4',5,5'-HxCB (169) were below the detection limit.

were detected in the soil samples from the Ishikari area. On the other hand, no contaminated compounds were detected in the soil samples from Rankoshi and Takko (Table 2). The PCB concentrations in the mice (pooled) from Ishikari were relatively higher than in mice from Rankoshi (Table 2). Fig. 3 shows the TEQ levels of the mice collected from Ishikari and Rankoshi. Whole body TEQ values of co-planar PCBs of the mice from Ishikari and Rankoshi were 6.8 pg/g lipid weight (wt.) and 1.8 pg/g lipid wt., respectively, and total non-dioxin like PCB levels in the whole body of the mice from Ishikari and Rankoshi were 18000 and 4400 pg/g lipid wt., respectively (Table 2 and Fig. 3). Ishiniwa *et al*. (2013) analyzed the DRC levels in the liver of large Japanese field mouse collected from an agricultural site in Niigata prefecture[9], and the concentration of total Co-PCBs was 5400 pg/g lipid wt., which was 3–13 times higher than that observed in our present study[9] (Table 2). No differences in fat content were observed between the mice from Ishikari and Rankshi; the fat amount of the mice ranged from 2.3 to 2.9%. Concentration of testosterone in the mice collected from Ishikari was significantly higher than those from Takko, but no significant difference was found between Ishikari and Rankoshi (Fig. 4).

Discussion

To our knowledge, this is the first study describing the contamination status of non-dioxin like-PCBs, DDTs and PAHs in the large Japanese field mouse. The species occurs widely in secondary forests and lowlands in Japan, and this species is also where waste incinerators (major source of dioxins) are located[9]. Previous studies have measured the concentration of dioxins in the whole body of the Japanese field mouse[25]. However, the accumulation levels of dioxins are lower in terrestrial wildlife than those in aquatic animals[12], and only a few studies describe the effects of the environmental contaminants on terrestrial animals. In this study, the levels of non-dioxin-like PCBs and Co-PCBs in the whole body of mice from Ishikari were higher than were those in the mice from

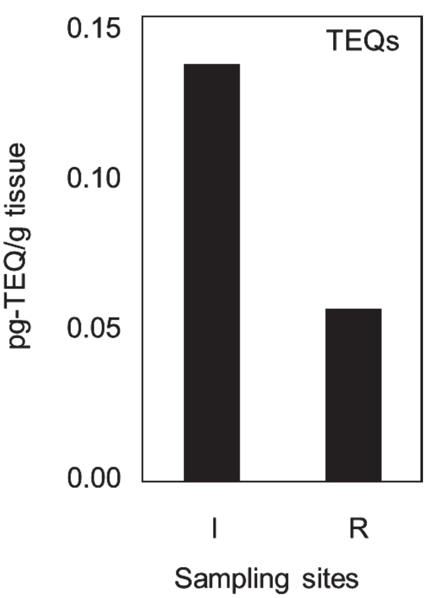

Fig. 3. Toxicity equivalence quantities (TEQs) levels of Co-planar polychlorinated biphenyls (PCBs) in the pooled male large Japanese field mice.

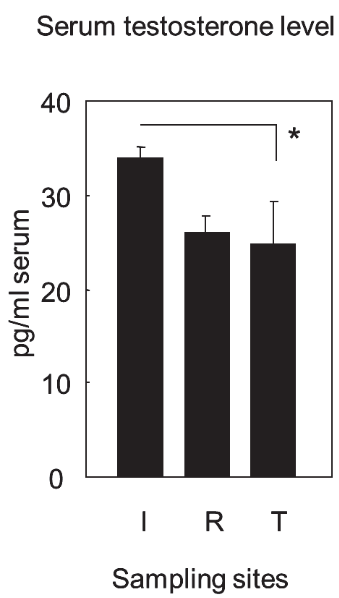

Fig. 4. Concentrations of serum testosterone in male large Japanese field mice. I denotes Ishikari and R, Rankoshi in Hokkaido, and T denotes Takko in Aomori prefecture. *: $p < 0.05$

Rankoshi. Moreover, PAHs that occur due to human activities were detected in the soil sample from Ishikari, indicating that Ishikari is the most contaminated region among the three areas analyzed, which in turn is dependent on the current population distribution. Ishiniwa *et al*. (2013) analyzed the levels of DRCs, and the Co-PCBs concentrations in the liver of large Japanese field mice collected from Niigata and Yamagata prefectures were higher[9] than those observed in this study. The findings, therefore, suggest that Ishikari, Rankoshi, and Takko may represent the non-contaminated regions of Japan although the liver samples were not analyzed in this study. A few studies have shown the accumulation levels of environmental pollutants such as POPs and metals in the wild wood mouse (*A. sylvaticus*), which is closely related to the large Japanese field mouse[2,6,16]. Borras and Nadal (2004) reported that *Apodemus sp.* could be used as a biomarker of genotoxicity caused by environmental carcinogens[3]. Rodents are common worldwide and are easy to catch; therefore, wild rodents could be used as biomarkers of terrestrial environmental pollution, and the contamination status of each region can be assessed.

The seasonal alterations in reproductive organs in the large Japanese field mouse are regulated by the hypothalamus, pituitary, testis or ovary axis[14]. A previous study showed that the size of the testis dramatically changes with the season[14]. During the breeding periods, the testis enlarges and the level of testosterone increases. The TW/BW ratios were calculated and the lowest value was found in the mice from Ishikari (Fig. 2). The results of this study suggest that the function of the testis in terms of the secretion of androgens decreased caused by biological and environmental factors including the exposure to Co-PCBs. However, the concentration of testosterone was the highest in mice collected from Ishikari compared to those from the other areas (Fig. 4). In TCDD-treated rat, increased in sperm mortality and abnormality caused by the exhaustion of the antioxidant defense system, which in turn impairs reproduction[5]. Moreover, it has been reported that prenatal testosterone levels in male rats exposed during pregnancy low-level exposure to DRCs increases the concentration or production of testosterone[8,10]. Therefore, POPs exposure is the one of the factor of the high levels of testosterone in the mice collected from the Ishikari area in addition to environmental and biological factors such as habitat density, age, regional difference of ending time of breeding period, temperature and/or food riches.

The present study indicates the one of the possibility of suppression of testicular function and reproductive ability in wild wood mice through continuous exposure to contaminants such as Co-PCB, although these mice showed low levels of contaminants. Further studies are needed to elucidate the toxic effects of these contaminants on reproductive functions and to reveal the mechanism of inducing toxicity in low-level exposures.

Acknowledgments

We thank Dr. Akio Kazusaka for contributing to the manuscript. This study was supported by Research Fellowships from the Japan Society for the Promotion of Science (JSPS) as Grant-in-Aid for Research Activity (No. 2589200103 (Start-up), 24248056 and 24405004) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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