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Histologic Changes in Thyroid Glands from Great Cormorant (*Phalacrocorax carbo*) in Tokyo Bay, Japan: Possible Association with Environmental Contaminants

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ABSTRACT: We compared morphologic changes in thyroid glands of great cormorants (*Phalacrocorax carbo*) from the Tokyo Bay and Lake Biwa areas in Japan with presence of residues of polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and coplanar polychlorinated biphenyls (Co-PCBs). Prominent morphologic changes in thyroid glands included increased density of small follicles and increased number of epithelial cells surrounding follicular lumens. The extent of morphologic changes in the thyroid gland was higher in cormorants captured from Tokyo Bay than in those captured from Lake Biwa. Increased thyroid change in cormorants from the Tokyo Bay area was associated with significantly higher levels of PCDFs and Co-PCBs. Thus, we suggest that morphologic changes in thyroid glands from the cormorants are associated with increased levels of dioxin contamination in Japan.

Key words: Dioxins, great cormorant, organochlorines, *Phalacrocorax carbo,* thyroid gland, Tokyo Bay, Japan.

It has been postulated that organochlorines (OCs) are associated with abnormalities in piscivores. These abnormalities include decreased reproductive success in free-ranging populations of fish-eating birds, such as cormorants and terns (Fox et al., 1991; Van den Berg et al., 1992; Yamashita et al., 1993). Functional and histopathologic changes have been demonstrated in thyroid glands in wild species, such as herring gulls (*Larus argentatus*) (Moccia et al., 1986), harbor porpoises (*Phocoena phocoena*) (Schumacher et al., 1993), mummichogs (*Fundulus heteroclitus*) (Zhou et al., 1999), and beluga whales (*Delphinapterus leucas*) (Guise et al., 1995). Organochlorine-exposed rats (Sewall et al., 1995b), harbor seals (*Phoca vitulina*) (Brouwer and Koeman, 1989), American kestrels (*Falco sparverius*) (Hoffman et al., 1996b; Smits et al., 2002), mallards (*Anas platyrhynchos*) (Fowles et al., 1997b), and Japanese quail (*Coturnix coturnix japonica*) (Grassle and Biessmann, 1982) had morphologic changes in thyroid follicles, immunosuppression, and drastic reduction of circulating retinol and thyroid hormones. These observations led to the suggestion that there is a causal link between the presence of OC residues in animals and morphologic and functional abnormalities of the thyroid gland.

Levels of OC residues were highest in great cormorants (*Phalacrocorax carbo*) among wild birds, such as silky chicken, common pheasant (*Phasianus colchicus*), rock pigeon (*Columba livia*), and large-billed crows (*Corvus macrorhyncho*), that have been studied in Japan (Iseki et al., 2000; Senthilkumar et al., 2002). The high levels found in great cormorants may be because cormorants entirely depend on fish for their diet and are at the top of the food chain. Moreover, cormorants have low activity of the cytochrome-450 family enzymes (Fossi et al., 1995). A previous study reported that total non-*ortho* coplanar equivalents were threefold higher in adult great cormorants collected from Shinobazu Pond (Tokyo Bay area, Japan; 139°E, 35°N) than those from the Lake Biwa area (136°E, 35°N) (Guruge et al., 2000). Therefore, the objectives of our study were to collect great cormorants from Tokyo Bay and Lake Biwa areas and

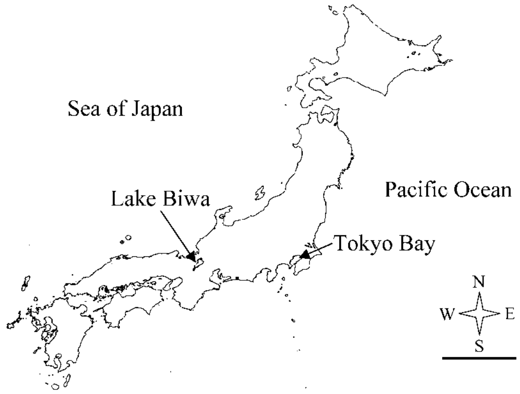

FIGURE 1. Location of Tokyo Bay and Lake Biwa where great cormorants were collected. Bar represents approximately 330 km.

compare histopathologic changes in the thyroid glands and levels of dioxin residues in birds from these areas.

Eighteen great cormorants were randomly shot in the Tokyo Bay area (eight juveniles, three adult males, seven adult females) in February and March 1999. Eleven great cormorants were similarly collected from Lake Biwa area (three juveniles, six adult males, two adult females) in February 1998 (Fig. 1). Thyroid glands were removed and fixed in 10% formalin. Tissues were routinely embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for quantitative histologic evaluation.

Pectoral muscles were removed from the birds and frozen at −20 C until analysis. Concentrations of polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and coplanar polychlorinated biphenyls (Co-PCBs) were analyzed by Miura Institute of Environmental Science (Hokujyo, Ehime, Japan) according to standard techniques (Ministry of Environment, 2003). Values of each chemical are expressed as 2-, 3-, 7-, 8-trichlorinated dibenzo-dioxins (TCDD) toxic equivalents (TEQs), while PCDDs, PCDFs, and coplanar PCBs were estimated using 2-, 3-, 7-, 8-TCDD toxic equivalency factors (TEF), as suggested by Van den Berg et al. (1998).

For quantitative histologic evaluation, 18 adult cormorants (10 cormorants from Tokyo Bay and eight cormorants from Lake Biwa) were used. Follicles in five fields (500×500 μm each) were counted in a randomly chosen portion in the largest section of thyroid gland under 100× magnification. Follicles touching the edge of the field were not included. Mean number of follicles from five fields is reported for each bird. To determine the number of follicular epithelial cells, 10 follicular lumens were randomly chosen per specimen under 400× magnification. The number of follicular epithelial cells surrounding each follicular lumen was counted and the data obtained for each bird were expressed as the number of cells per 100 μm^2.

Because there was a wide difference in thyroid morphology between birds from the two sites, specifically in follicular size, number of follicles, and number of follicular epithelial cells, thyroid morphology was classified as follows. Thyroid glands of cormorants that had a mean of more than 30 follicles per specimen and a mean of more than 40 follicular epithelial cells around follicular lumens per specimen were classified as the extensive hyperplasia group. Cormorants that had mean values less than the extensive hyperplasia birds were classified as the mild hyperplasia group.

Differences between means were evaluated by the Mann-Whitney *U*-test. A value of $P<0.05$ was considered statistically significant.

Residues of PCDDs, PCDFs, and Co-PCBs were not different between males and females in populations from Tokyo Bay and Lake Biwa (data not shown). However, there were differences between adults and juveniles (Fig. 2). All chemicals analyzed were higher in adults as compared with juveniles; however, only PCDFs, non-*ortho* Co-PCBs, mono-*ortho* Co-PCBs, and total Co-PCBs levels were significantly different ($P<0.05$).

There were no significant inflammatory, degenerative, or necrotic changes in thy-

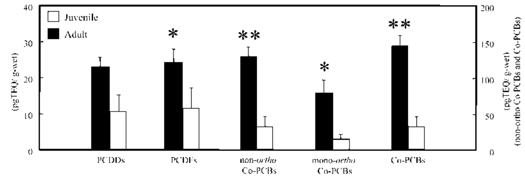

FIGURE 2. Residues of PCDD, PCDFs, and coplanar PCBs in juvenile (□, *n*=5) and adult (■, *n*=15) great cormorants. Results are expressed as mean toxic equivalents (TEQ)±SEM and differences are indicated: *=*P*<0.05, **=*P*<0.01. Toxic equivalency factor values of polychlorinated dibenzo-dioxins (PCDD), polychlorinated dibenzo-furans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) are from Van den Berg et al. (1998).

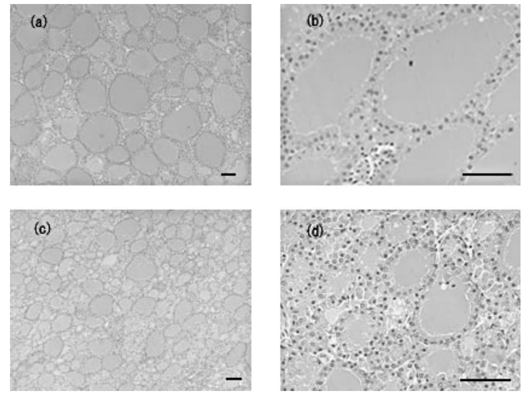

FIGURE 3. Morphologic changes in thyroid glands from great cormorants. Mild (a, b) and extensive (c, d) changes are shown. (b and d) High magnifications of (a) and (c), respectively. Note that (c) and (d) have a greater number of small follicles and more follicular epithelial cells than those found in (a) and (b), respectively. Bars = 50 μm.

roid tissues from the cormorants. However, small follicles and increased numbers of follicular epithelial cells were found (Fig. 3). Average number of thyroid follicles in selected fields of thyroid glands from adult cormorants from the Tokyo Bay area was significantly ($P<0.05$) higher than that of birds from the Lake Biwa collection (44.1±5.0; $n=9$ versus 24.4±5.0; $n=6$). Average number of follicular epithelial cells in thyroid glands of adult cormorants from Tokyo Bay collection tended to be higher than that from birds from the Lake Biwa collection, although the difference was not significant (57.1±7.8; $n=6$ versus 35.4±8.6; $n=6$). There were no statistical differences in the numbers of follicular epithelial cells per 100 μm^2 of colloid in birds from the Tokyo Bay area compared with those from the Lake Biwa area.

On further analysis, seven of nine cormorants from the Tokyo Bay collections had extensive hyperplastic changes in the thyroid, while two cormorants had mild hyperplastic changes. In comparison, in birds from the Lake Biwa collections, one of six cormorants had extensive hyperplastic changes, while five cormorants had mild hyperplastic changes. The levels of dioxin residues in the birds with extensive and mild hyperplastic changes are shown in Figure 4. Concentrations of non-*ortho* Co-PCBs (156.4±19.3 versus 94.6±14.8 pg TEQ/g-wet) and Co-PCBs (177.8±22.6 versus 104.1±17.7 pg TEQ/g-wet) occurred in the group with extensive hyperplastic change and mild hyperplastic change, respectively, and these differences were significant. Concentrations of other dioxins in the group with extensive histologic change tended to be higher as compared with the mildly affected group, but these differences were not statistically significant (Fig. 4).

Concentrations of dioxins in adult cormorants are shown in Figure 5. All dioxin residues except PCDDs tended to be higher in birds collected from the Tokyo Bay area compared with Lake Biwa; how-

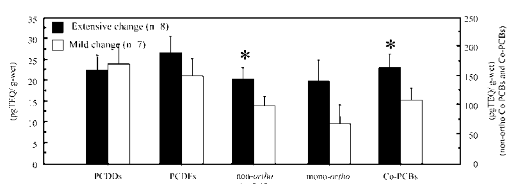

FIGURE 4. Residues of polychlorinated dibenzo-dioxins (PCDD), polychlorinated dibenzo-furans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) in cormorants (■, *n*=8) with extensive thyroid change and in cormorants (□, *n*=7) with mild thyroid change. Results are expressed as toxic equivalent (TEQ) mean+SEM. Toxic equivalency factor values of PCDDs, PCDFs, and coplanar PCBs are from Van den Berg et al. (1998).

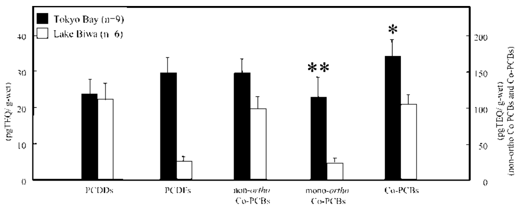

FIGURE 5. Residues of polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) in adult cormorants from the Tokyo Bay area (n=9) and Lake Biwa area (n=6). Results are expressed as mean toxic equivalents (TEQ)±SEM and differences are indicated: *=$P<0.05$, **= $P<0.01$ Toxic equivalency factors values of PCDD, PCDFs, and coplanar PCBs are from Van den Berg et al. (1998).

ever, only mono-*ortho* Co-PCBs and Co-PCBs levels were significantly different.

Histopathologic analysis demonstrated that thyroid glands of great cormorants collected from Tokyo Bay area had thyroid follicles of reduced size, a higher density of follicles, and an increased number of follicular epithelial cells as compared with those in cormorants from the Lake Biwa collection. We also demonstrated that dioxin residues in pectoral muscles of great cormorants are high in Tokyo Bay birds as compared with those from the Lake Biwa area. In addition, all dioxin residues analyzed were higher in adults than in juveniles, indicating the age-dependent accumulation of dioxins. This is the first demonstration of a clear difference in thyroid morphology in cormorants from Tokyo Bay and Lake Biwa correlated with concentrations of OC residues. These results confirm the findings of Guruge et al. (2000), who reported that adult cormorants from Shinobazu Pond located in Tokyo had significantly higher concentrations of the sum of non-, mono- and di-*ortho* Co-PCB congeners in their liver (40,000 [18,000–58,000] ng/g wet weight) than those in chicks (940 [780–1,100] ng/g wet weight) and juveniles (9,100 [800–15,000] ng/g wet weight).

Field studies have found a clear association between exposure to environmental contaminants and alterations in thyroid gland morphology in free-ranging populations of wildlife. Thyroid gland mass of herring gulls from the Great Lakes basin of eastern North America was greater than that in herring gulls from the Bay of Fundy (Canada) and the thyroid glands were microfollicular (Moccia et al., 1986). The levels of OCs in herring gulls from the Great Lakes were the highest reported anywhere in the world for fish-eating birds at that time (Keith and Grucky, 1970). Nestling American kestrels exposed to OCs exhibited decreased follicle size, thinning of colloid, and collapse of empty follicles (Hoffman et al., 1996a). Sprague-Dawley rats treated with TCDD had small follicles filled with pale and sometimes clumped colloid and were lined by tall cuboidal follicular epithelial cells (Sewall et al., 1995a), increased thyroid weight, decreased colloid area, and an increase in the number of small follicles (Nishimura et al., 2002). Wistar rats fed PCBs developed extensive hyperplasia and hypertrophy of follicular cells accompanied by colloid depletion (Akoso et al., 1982). These changes in thyroid morphology of animals exposed to OCs in laboratories are similar to morphologic changes of the thyroid glands observed in great cormorants in the present study. On the other hand, PCB (Aroclor 1254) -treated adult male mallards had significantly increased thyroid weights and reduced plasma total triiodothyronine concentrations. But there were no significant histopathologic lesions in the thyroids except increased vacuolization and slightly increased thyroidal colloid with 500 mg PCB/kg treatment. Based on previous reports and the morphologic changes in thyroid glands we found, it is likely that there is an interaction between the residues of dioxins and adverse effects on thyroid morphology in the great cormorant.

Thyroid hormones regulate basal metabolism and influence the immune system. Thyroid hormones increase proliferation of lymphocytes (Kruger, 1996); mallards with hypothyroidism induced by treatment with methimazole had reduced cell-mediated immunity (Fowles et al., 1997a). Therefore, abnormal changes in the thyroid glands of great cormorants could lead to dysfunction of thyroid hormone-associated endocrine and immune system. Exposure of American kestrels to PCBs caused decreased plasma concentration of thyroid hormones and immunomodulation of cell-mediated and humoral immune responses (Smits et al., 2001) and elicited a greater response to the phytohemagglutinin skin test representing T lymphocyte proliferative potential (Smits et al., 2002).

In summary, our results suggest a possible connection between high concentrations of some dioxin residues and increased density of follicles together with an increased number of follicular epithelial cells in great cormorants. Moreover, the bioaccumulation of dioxin residues, which likely caused the thyroid lesions, appeared higher in birds from the Tokyo Bay area in comparison with those from the Lake Biwa area. Thus, we suggest that histologic examination of the thyroid gland combined with measurement of plasma thyroid hormone levels could be used for evaluation of OC exposure of wildlife once validated.

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