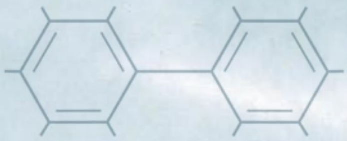
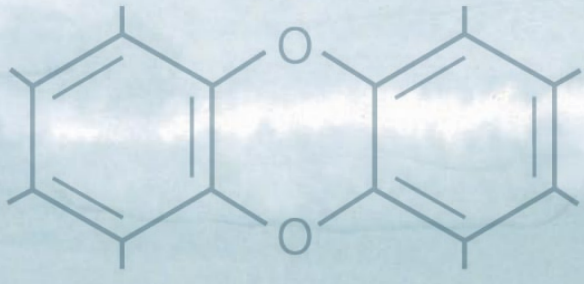
Survey on the State of Dioxins Accumulation in Wildlife (1998-2007)

# The Accumulation of Dioxins in the Japanese Wildlife



Environmental Risk Assessment Office
Environmental Health Department
Ministry of the Environment, Japan



# What are Dioxins ?

Dioxins are compounds generated as unintended by-products during processes when heat is applied to substances containing carbon, oxygen, hydrogen and chlorine, most often during waste incineration.

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) are all referred to as dioxins.

Dioxins are known to gradually decompose on exposure to sunlight, but they show poor water solubility, and do not react easily with other chemical substances, as a result of which they tend to remain in the environment for a long time.

Not only by human activities including waste incineration, electric steel-making furnaces, cigarette smoke and automobile exhaust, dioxins are also produced by such natural events as forest fires and volcanic activity.

Some reports indicate that dioxins may have accumulated in bottom sediments in the water environment owing to the past use of PCBs and some types of agricultural chemicals, which contain dioxins as impurities.

The behavior of dioxins in the environment is not fully known. For example, dioxins in the air may absorb on particulate matter, fall to the ground and pollute soil and water. It is considered that over long periods of time these dioxins, together with those released into the environment via various other pathways, ultimately accumulate in aquatic sediments and enter the food chain when ingested by plankton and fish, thereby accumulating in various organisms in the biota. Dioxins are also very persistent in the body of organisms, and accumulate mainly in the adipose tissue.

Dioxins have been monitored as toxic substances that can cause cancer and defects in fetuses.


Figure 1  Behavior of dioxins within the ecosystem

# Dioxins Contamination in Japanese Wildlife

## Monitoring of Dioxins pollution in Wildlife

To understand the status of accumulation of dioxins in Japanese wildlife, Ministry of the Environment, Japan surveyed dioxins levels in 1,428 specimens from 18 species (or group of species) of Japanese wildlife from fiscal year 1998 to 2007. The Ministry also investigated dioxins in 258 specimens belonging to 5 species (or group of species) as part of the Survey on the Impacts of Endocrine Disruptors on Wildlife conducted during the same period.

## Q1. Was the occurrence of dioxins contamination confirmed in wildlife?

Yes. Certain species accumulated at much higher level of dioxins than human beings, but overall, dioxins accumulation in Japanese wildlife was found to be at a low level compared with those in the regions such as the Great Lakes in North America where anomalies were found in wild animals.

⇨ **See the following page for details: “Concentration of dioxins in Japanese wildlife” (p.8)**

## Q2. Which species are highly contaminated?

The following wildlife were found to be highly contaminated:

- Animals at the top predator in the food chain
- Animals principally feeding on fish
- Male animals in old age

Concentrations were varied even in the same individual according to organs and tissues, with certain species showing high concentrations in the liver.

Great cormorant

Hodgson’s hawk-eagle

⇨ **See the following pages for details: “Differences among species” (p.9)**
**“Differences in the same species” (p.10)**

## Q3. Is Japanese wildlife suffering any adverse impacts from dioxins?

This survey turned up no defects or other apparent impacts. However, detailed investigation revealed internal responses to dioxins accumulation in wildlife that are not visible to the naked eye.

⇨ **See the following page for details: “Adverse effects” (p.12)**

## Q4. Is the concentration of dioxins in Japanese wildlife declining?

No. The concentration of dioxins in the air and aquatic environments is declining as a result of initiatives to stem emissions, including the creation of a Dioxin Emission Inventory and imposition of emission limits on specific facilities under the Law Concerning Special Measures against Dioxins. However, dioxins levels of certain environments such as bottom sediment have not declined, and concentrations in wildlife have yet to show any decrease.

⇨ **See the following page for details: “Temporal trends” (p.13)**

Finless porpoise

# Contents



Photo by the Shimonoseki Marine Science Museum “Kaiyokan” for finless porpoise; Hitoshi Imai, Tomonari Nakajima
Illustration: Yuko Horiki

# 1. Overview

The Survey on the State of Dioxins Accumulation in Wildlife (1998–2007) was conducted with the following goals in mind:

- To ascertain whether the results obtained from monitoring the state of wildlife could serve as indicators for the assessment of impacts of dioxins on human beings
- To gather basic data for the assessment of impacts of dioxins on wildlife itself

### Survey process

- In fiscal 1997, a Research Team to Survey the State of Pollution by Dioxins in Wildlife was established, and a Manual for Surveys of Wildlife Pollution by Dioxins (July, 1998) was prepared.
- In 1998, a nationwide survey of a broad range of species was conducted.
- In 1999, the number of species was narrowed down, and the survey continues.
- In 2000, survey of impacts was started.
- The manual was revised in line with advances in analytical methods and newly established quality assurance guidelines, and a new edition of the Dioxins Survey Manual for Wildlife of Japan (September, 2002) that also included methods of impacts survey was compiled.
- The data used in this brochure also include results of dioxin concentration and impact survey obtained as part of the Survey on the State of Impacts of Endocrine Disruptors on Wildlife conducted during the same period by the Ministry of the Environment.

# 2. Materials and Method

## 2-1 Wildlife samples

- The 1998 survey focused on relatively widely distributed species and species at various levels in the food chain. The target species were selected among fish, amphibians, birds and mammals.
- Based on the results of 1998 survey, surveys from 1999 onwards focused on the living environment of wildlife. Animals were classified into groups of those living mainly in the open sea, those living mostly in coastal waters, those living both coastal marine and land area, and those living mainly on land. Target species were selected from each group, with consideration of selecting species that could be surveyed on a sustained basis.
- Specimens of the target species were obtained through trapping with capture permission for academic research (e.g. large Japanese field mice), wildlife control (e.g. great cormorants and jungle crows), strandings (e.g. finless porpoises), bycatch (e.g. harbor seals), roadkills (e.g. raccoon dogs) and other routes.
- Specimens were collected from various parts of Japan in 1998, but from 1999 onwards, they were collected from the same locations each year. Consideration was also given that selections of sampling location enable comparison between different environments, such as rural and urban environments (Figures 2 to 5).
- The tissues analyzed were chosen according to the attributes of each species, and included adipose tissue, muscle and liver. In 1998, mixed tissue specimens were used, but from 1999 onwards, analyzed samples were consisted of single tissue.

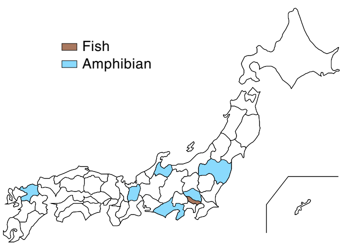



Figure 2.
Fish and amphibian survey areas

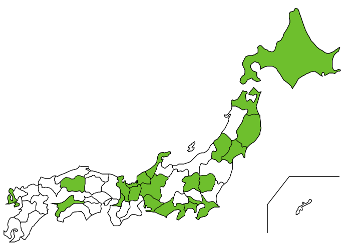


Figure 3.
Bird survey areas

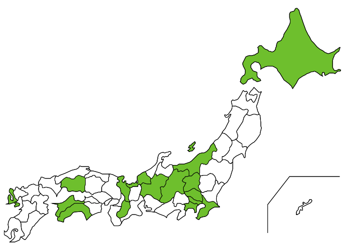


Figure 4.
Terrestrial mammal survey areas

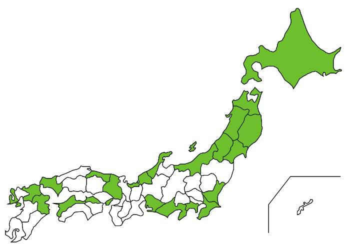
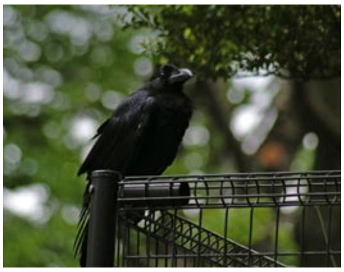


Figure 5.
Marine mammal survey areas

## 2-2 Items analyzed

- Dioxins (seven PCDDs, 10 PCDFs, 12 Co-PCBs) were measured.
- Survey of impacts were conducted on species for which collection of required tissue specimens was possible, namely great cormorant, jungle crow, black kite, and large Japanese field mouse. Liver drug metabolizing enzyme activity, and blood thyroid hormone and sex steroid hormone concentrations were measured, and thyroid glands, gonads and other major organs were histopathologically examined (See the supplementary information on p.17 “Adverse effects” for details of the survey of impacts).


Large Japanese field mouse


Jungle crow

## 2-3 Expression of analytical data

- When considering the impacts of dioxins on organisms, in addition to concentration, figures referred to as toxic equivalents (TEQs) are used. TEQs are calculated by expressing the toxicity of dioxins relative to that of 2,3,7,8-TCDD, the most toxic dioxin, and adding up the total.
- Results of analysis may be displayed either in terms of the weight of the tissue specimen measured (wet weight), or of the weight of the fat content of the specimen (fat weight).
- In this brochure, pgTEQ/g wet is used as the TEQ unit for wet weight, and pgTEQ/g fat as the TEQ unit for fat weight.


Black kite

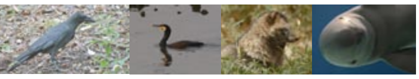
Table 1 **Concentrations of dioxins in Japanese wildlife (pgTEQ/g fat)**

| Taxon | Species | Specimen | N | Average | Standard deviation | Median | Range |
|---|---|---|---|---|---|---|---|
| Reference | Japanese People | Blood | 1,656 | 20 | 14 | 16 | 0.64 - 120 |
| Fish | Carp | Muscle | 48 | 160 | 110 | 130 | 27 - 630 |
| Amphibians | Frogs | Whole body | 101 | 310 | 190 | 250 | 43 - 950 |
| Birds | Feral pigeon | Mixed (incl. liver) | 15 | 50 | 50 | 18 | 9.0 - 150 |
| | Jungle crow | Muscle | 33 | 21 | 13 | 18 | 4.2 - 66 |
| | | Liver | 66 | 79 | 67 | 58 | 6.0 - 360 |
| | Black kite | Adipose tissue | 17 | 540 | 520 | 330 | 41 - 1,600 |
| | | Muscle | 98 | 670 | 1,500 | 300 | 31 - 14,000 |
| | | Liver | 57 | 760 | 1,300 | 420 | 74 - 9,200 |
| | | Mixed (adipose tissue, muscle) | 20 | 560 | 520 | 380 | 78 - 2,300 |
| | Raptors [1] (excluding black kite) | Muscle | 33 | 3,500 | 5,300 | 1,400 | 130 - 27,000 |
| | | Liver | 18 | 3,800 | 9,000 | 790 | 86 - 38,000 |
| | | Mixed (adipose tissue, muscle) | 4 | 270 | 180 | 250 | 81 - 510 |
| | | Egg | 11 | 2,300 | 2,100 | 1,400 | 230 - 8,100 |
| | Great cormorant | Adipose tissue | 6 | 3,600 | 3,300 | 3,000 | 180 - 8,400 |
| | | Muscle | 194 | 2,300 | 2,900 | 1,500 | 23 - 24,000 |
| | | Liver | 128 | 2,900 | 4,300 | 1,800 | 39 - 41,000 |
| | | Egg | 90 | 2,900 | 1,600 | 2,600 | 850 - 12,000 |
| Terrestrial mammals | Large Japanese field mouse | Body [3] | 167 | 61 | 56 | 44 | 3.6 - 490 |
| | | Liver | 66 | 2,700 | 2,800 | 1,900 | 110 - 16,000 |
| | | Mixed (body, liver) | 37 | 880 | 720 | 720 | 41-2,700 |
| | Japanese monkey | Adipose tissue | 16 | 40 | 47 | 19 | 5.6 - 170 |
| | | Muscle | 4 | 54 | 84 | 18 | 0.85 - 180 |
| | | Mixed (adipose tissue, muscle) | 10 | 12 | 13 | 5.7 | 1.2 - 43 |
| | | Mixed (incl. liver) | 10 | 19 | 22 | 5.7 | 1.4 - 56 |
| | Raccoon dog | Adipose tissue | 71 | 56 | 87 | 22 | 9.8 - 450 |
| | | Muscle | 10 | 230 | 260 | 97 | 20 - 650 |
| | | Liver | 24 | 290 | 160 | 280 | 56 - 810 |
| | | Mixed (incl. liver) | 6 | 32 | 27 | 25 | 14 - 87 |
| | Bears | Adipose tissue | 10 | 0.51 | 0.61 | 0.30 | 0.21 - 2.2 |
| | | Mixed (adipose tissue, liver) | 6 | 0.62 | 0.61 | 0.35 | 0.26 - 1.8 |
| | Sika deer | Kidney; Mixed (kidney, adipose tissue) | 48 | 27 | 46 | 14 | 3.1 - 310 |
| | | Liver; Mixed (liver, adipose tissue) | 30 | 44 | 40 | 34 | 7.3 - 200 |
| | Wild boar | Muscle | 7 | 6.3 | 7.3 | 3.4 | 2.9 - 23 |
| Marine mammals | Seals | Adipose tissue | 26 | 11 | 4.3 | 11 | 4.8 - 25 |
| | Finless porpoise | Blubber [4] | 90 | 56 | 46 | 41 | 7.7 - 280 |
| | | Muscle | 23 | 40 | 31 | 32 | 5.1 - 110 |
| | Stejneger's beaked whale | Blubber | 37 | 95 | 48 | 83 | 10 - 200 |
| | | Muscle | 19 | 110 | 88 | 60 | 38 - 380 |
| | Other whales [2] | Blubber | 16 | 64 | 45 | 59 | 3.7 - 150 |

1) Including Hodgson's hawk eagle, goshawk, peregrine falcon and owls.
2) Including dolphins and baleen whales.
3) For this survey, we use the word "body" to refer to large Japanese field mouse specimens after skinning and removal of internal organs, head, tail, and extremities.
4) Blubber is a fat-storing dermal tissue unique to cetaceans.

**Notes**

* Calculated by totaling the survey results of multiple years and locations.
* Includes results of analysis of multiple specimens from the same individual, e.g. adipose tissue and liver, muscle and liver, body and liver.





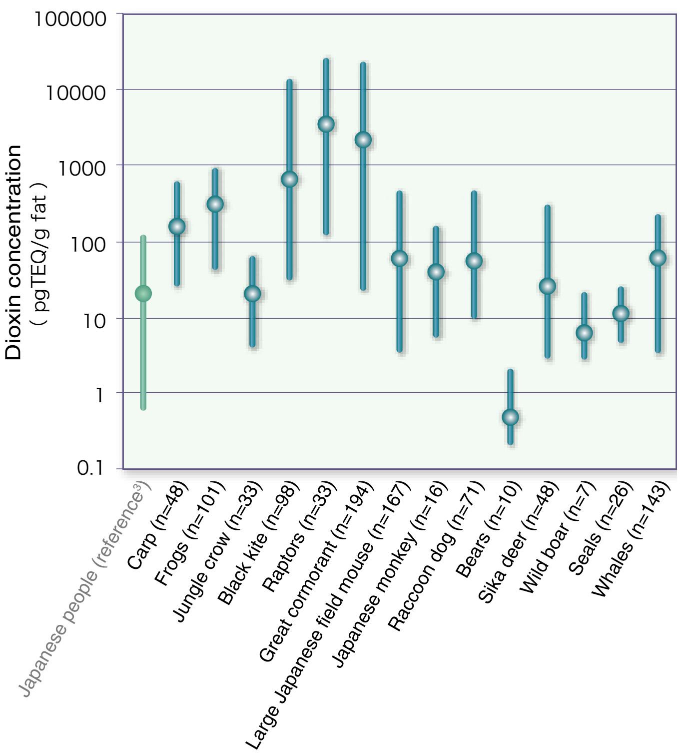
# 3. Results

## 3-1 Concentration of dioxins in Japanese wildlife

The concentration of dioxins found in Japanese wildlife collected from 1998 to 2007 fell in the range of 0.21–41,000 pgTEQ/g fat (Table 1, Figures 6, 7).

Regarding wildlife overseas, it has been reported[1] that bald eagle carcasses collected in 2000 in the USA had concentrations of 11,000–380,000 pgTEQ/g fat in muscle, and 1,500–150,000 pgTEQ/g fat in their livers. Eggs of waterfowl inhabiting the Great Lakes region have also been reported to show concentrations of 1,250–2,700 pgTEQ/g wet[2].

Compared with wildlife found to have abnormalities in these overseas locations, the concentration of dioxins in Japanese wildlife is on the whole low (Figures 6, 7). However, dioxin concentrations in the bodies or eggs of great cormorants and raptors are 10–100 times higher than that of Japanese people or captive raptors eating food provided by people.



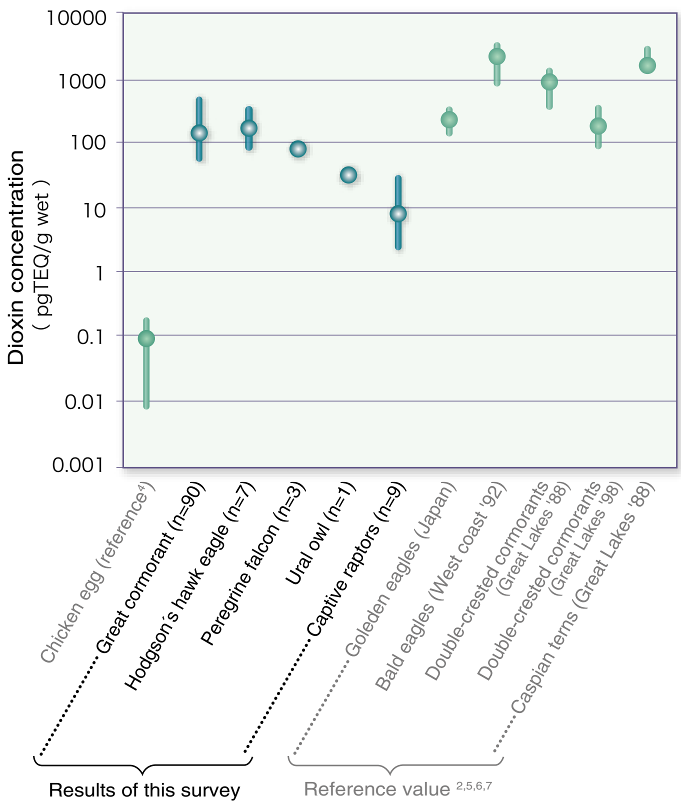




Figure 6
Concentrations of dioxins in Japanese wildlife (maximum – average – minimum)
(specimens used: adipose tissue, muscle or similar tissue, blood in the case of humans: fat weight)

Figure 7
Concentrations of dioxins in eggs of Japanese bird species
(maximum – average – minimum)
(specimens: eggs: wet weight)

## 3-2 Differences among species

The survey revealed differences in dioxin concentrations among species, with higher-order predators in the food web such as great cormorants and raptors showing higher concentrations (Figure 6).

Dioxin concentrations were derived in large part from Co-PCBs. The ratio of Co-PCBs was particularly high in marine mammals and birds (such as great cormorants) that eat mainly fish, and the same species exhibited higher overall dioxin concentrations (Figure 8, Table 2).

Co-PCBs are a type of PCB with comparable level of toxicity as PCDDs and PCDFs. They are thought to be derived not only from waste incineration, but also from the residues of PCBs manufactured and used up to the 1970s or leakage into the environment of stored PCBs. PCBs are known to disperse in the atmosphere more easily than PCDDs and PCDFs, and to accumulate in high concentrations in coastal fish species. They accumulate as a result in a wide variety of organisms, and particularly so in fish-eating species.

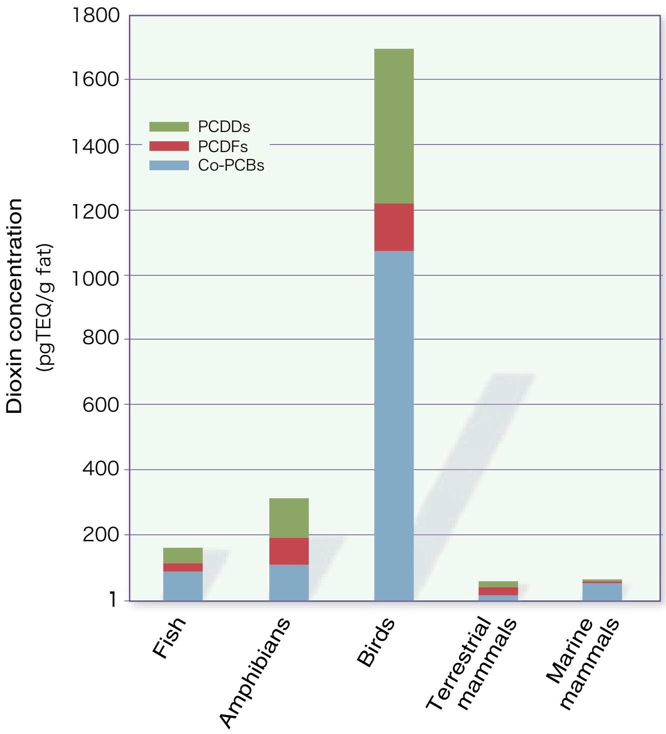



Figure 8
Dioxin concentrations in different animal species, and Co-PCBs ratios
(specimens composed of adipose tissue, muscle or similar tissues only)

Table 2 **Dioxin concentrations (specimens composed of adipose tissue, muscle or similar tissues only)**

| | | Fish | Amphibians | Birds | Terrestrial mammals | Marine mammals |
|---|---|---|---|---|---|---|
| Number of individuals surveyed | | 48 | 101 | 387 | 287 | 171 |
| Dioxin concentrations (pgTEQ/g fat) | Average | 160 | 310 | 1,700 | 60 | 62 |
| | Standard deviation | 110 | 190 | 2,900 | 85 | 68 |
| | Median | 130 | 250 | 640 | 35 | 45 |
| | Range | 27 - 630 | 43 - 950 | 4.2 - 27,000 | 0.21 - 650 | 3.7 - 680 |
| Co-PCBs ratio in the concentration (%) | Average | 54 | 34 | 63 | 34 | 84 |
| | Standard deviation | 8.5 | 9.4 | 21 | 21 | 10 |
| | Median | 54 | 34 | 70 | 36 | 85 |
| | Range | 28 - 74 | 9.5 - 53 | 7.0 - 95 | 3.7 - 95 | 6.9 - 99 |

## 3-3 Differences in the same species

### Gender difference and age-dependent accumulation

Variations in dioxin concentrations according to age and sex were observed within the same species. Concentrations generally appeared to increase with age (Figure 9).

Also in finless porpoises, large Japanese field mice and great cormorants, concentrations were statistically significantly lower in adult females than adult males (Tables 3 to 5, Figures 10 to 12). This is considered to be due to the elimination of dioxins through birth, lactation and egg-laying in the case of females.

Table 3 **Gender difference of dioxin concentrations in finless porpoises (Inland Sea)**

| | | Adult male | Adult female | Immature |
|---|---|---|---|---|
| Number of individuals surveyed | | 13 | 3 | 49 |
| Blubber dioxin concentration (pgTEQ/g fat) | Average | 95 | 27 | 47 |
| | Standard deviation | 71 | 7.6 | 27 |
| | Median | 68 | 26 | 38 |
| | Range | 30 - 280 | 21 - 36 | 10 - 140 |

Table 4 **Gender difference of dioxin concentrations in large Japanese field mice (Saitama Prefecture)**

| | | Adult male | Adult female | Immature |
|---|---|---|---|---|
| Number of individuals surveyed | | 34 | 42 | 24 |
| Body dioxin concentration (pgTEQ/g fat) | Average | 74 | 46 | 77 |
| | Standard deviation | 43 | 48 | 68 |
| | Median | 73 | 26 | 43 |
| | Range | 44 - 150 | 36 - 240 | 28 - 260 |

Table 5 **Gender difference of dioxin concentrations in great cormorants (Shiga Prefecture)**

| | | Adult male | Adult female | Juvenile |
|---|---|---|---|---|
| Number of individuals surveyed | | 29 | 28 | 14 |
| Muscle dioxin concentration (pgTEQ/g fat) | Average | 3,200 | 1,800 | 620 |
| | Standard deviation | 2,000 | 1,400 | 720 |
| | Median | 2,400 | 1,500 | 340 |
| | Range | 950 - 10,000 | 590 - 6,900 | 90 - 2,800 |

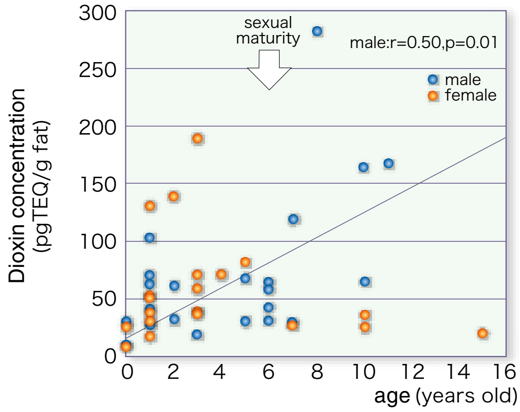



Figure 9 Age and dioxin concentrations of finless porpoises (Inland Sea, blubber)

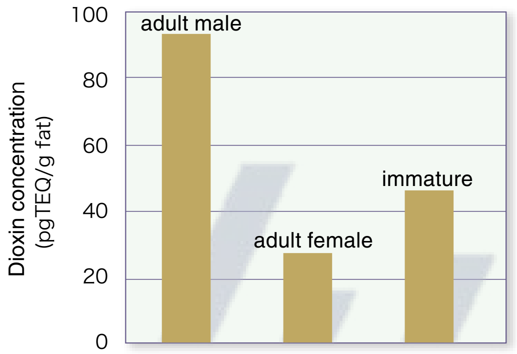



Figure 10 Dioxin concentrations in finless porpoises (average)

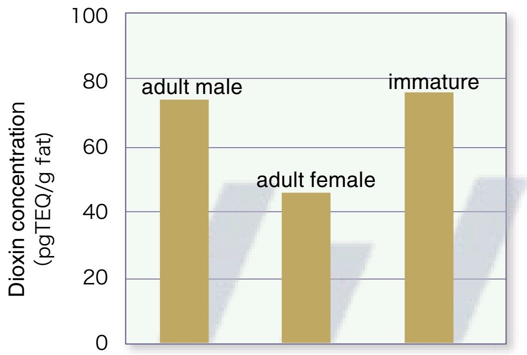



Figure 11 Dioxin concentrations in large Japanese field mice (average)

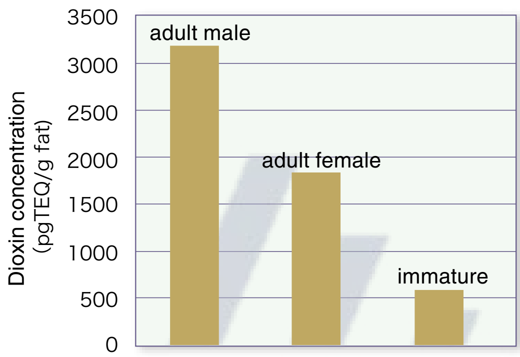



Figure 12 Dioxin concentrations in great cormorants (average)

## Regional differences

Investigation of black kites from both coastal and inland regions revealed a statistically significant difference between the regions (Table 6, Figure 13). Also, investigation of jungle crows and large Japanese field mice from rural and urban locations revealed statistically significant higher dioxin concentrations in urban locations (Table 7, Figure 14). These differences are thought to reflect differences in dioxin concentrations in food sources (i.e. exposure differences) derived from differences in environmental concentrations.

Table 6 **Dioxin concentrations in black kites from different regions**

| | | Inland area | Coastal area |
|---|---|---|---|
| Number of individuals surveyed | | 70 | 28 |
| Muscle dioxin concentration (pgTEQ/g fat) | Average | 860 | 200 |
| | Standard deviation | 1,700 | 130 |
| | Median | 440 | 190 |
| | Range | 53 - 14,000 | 31 - 560 |

Table 7 **Dioxin concentrations in jungle crows from different regions**

| | | Urban area | Rural area |
|---|---|---|---|
| Number of individuals surveyed | | 23 | 10 |
| Muscle dioxin concentration (pgTEQ/g fat) | Average | 23 | 14 |
| | Standard deviation | 15 | 4.5 |
| | Median | 19 | 14 |
| | Range | 4.2 - 66 | 9.1 - 20 |

## Hepatic sequestration

In species for which dioxin concentrations in liver and adipose tissue or muscle of the same individual were measured, liver dioxin concentrations tended to be higher, indicating hepatic sequestration. When the liver concentration is divided by the concentration in adipose tissue, muscle, or similar tissue, almost all species showed a value of over 1.0, with large Japanese field mice and raccoon dogs in particular showing marked hepatic sequestration of about 100 times and 17 times respectively (Figure 15).

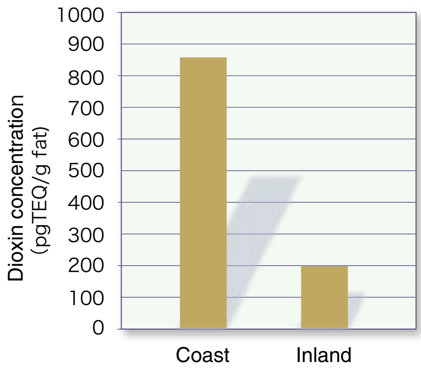



Figure 13 Dioxin concentrations (average) in black kites

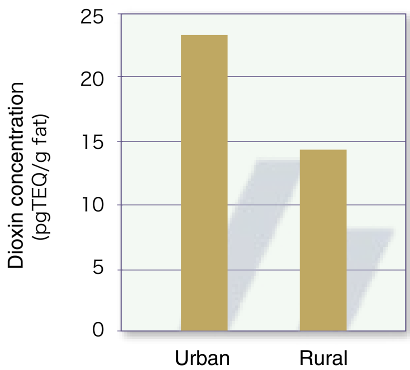
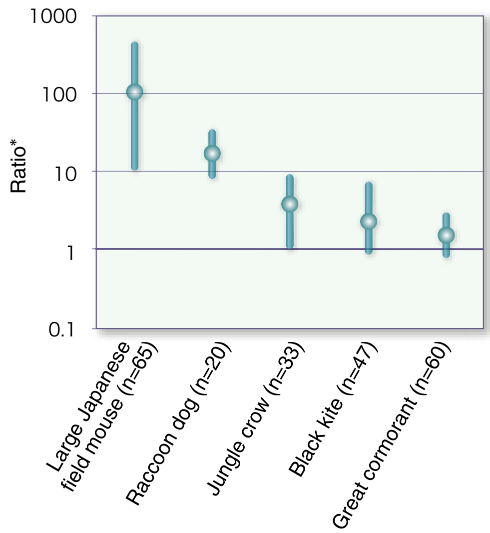



Figure 14 Dioxin concentrations (average) in jungle crows




* The value obtained from dividing the liver concentration by the concentration in adipose tissue, muscle, or similar tissue.

Figure 15 Hepatic sequestration ratios (maximum – average – minimum)

## 3-4 Adverse effects

Histopathological examination revealed abnormalities in thyroid gland and gonadal tissues of some individuals, but a correlation between these abnormalities and dioxin concentrations was not found.

In great cormorants and jungle crows, a statistically significant positive correlation was found between liver dioxin concentrations and drug-metabolizing enzyme activity (Figure 16), suggesting that dioxins were inducing drug-metabolizing enzyme activity.

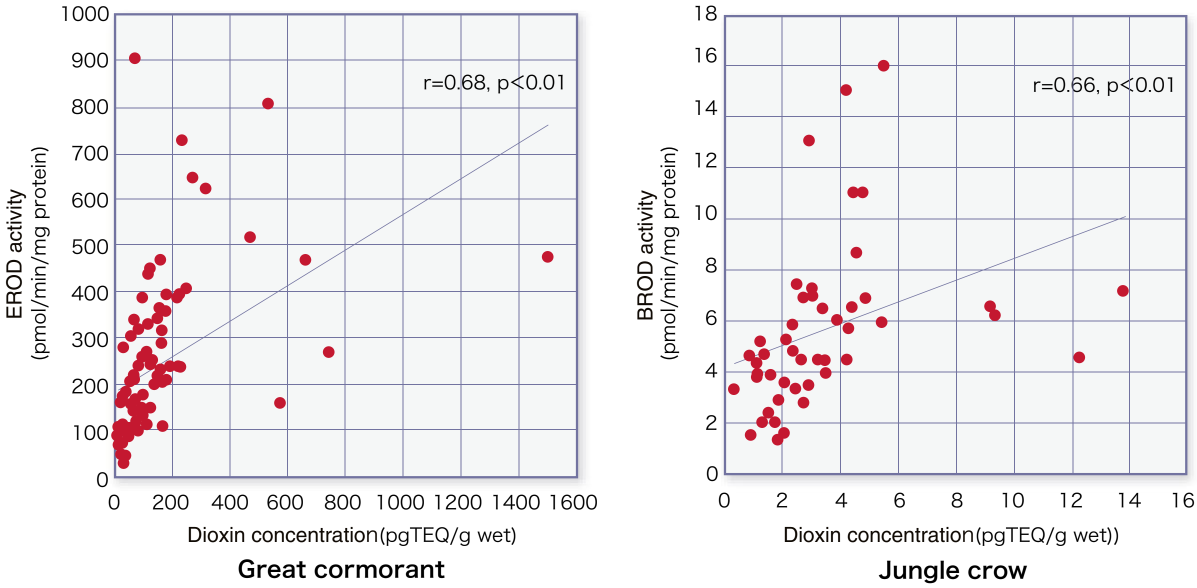



Figure 16 Relationship between liver drug-metabolizing enzyme activities and dioxin concentrations

Measurement of thyroid hormone concentrations in peripheral blood also revealed a statistically significant negative correlation with dioxin accumulation levels in great cormorants and other species (Figure 17).

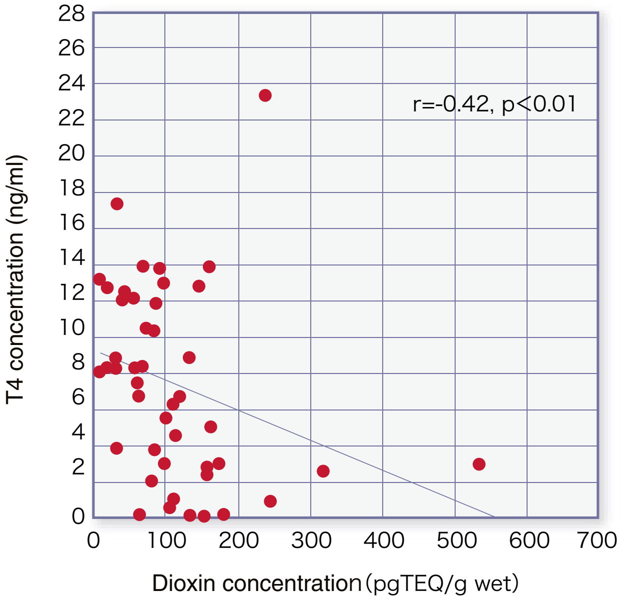



Figure 17 Relationship between great cormorant liver dioxin concentrations and blood thyroid hormone levels

## 3-5 Temporal trends

The amount of dioxins being released into the environment is declining in response to measures to curb dioxin emissions, and dioxin concentrations in the air, water and other media are declining as a result (Figures 18 and 19). However, there are environmental media such as bottom sediments in which dioxin concentrations are not declining, and no clear declining trend of dioxin concentrations in the wildlife was observed (Figure 20). This suggests that the decline in dioxin emissions will not be easily reflected in wildlife, and will need time to take effect.

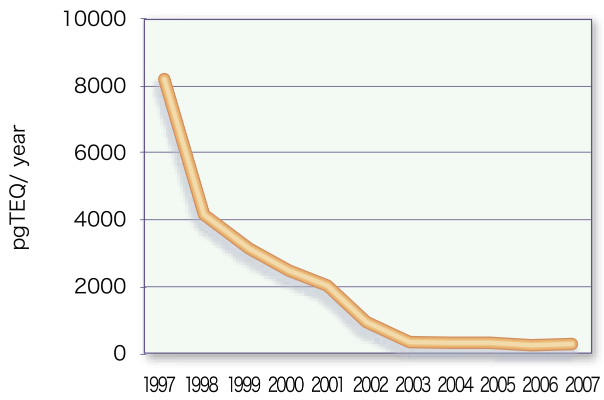



Figure 18 Temporal trends of total dioxin emissions (average) in Japan

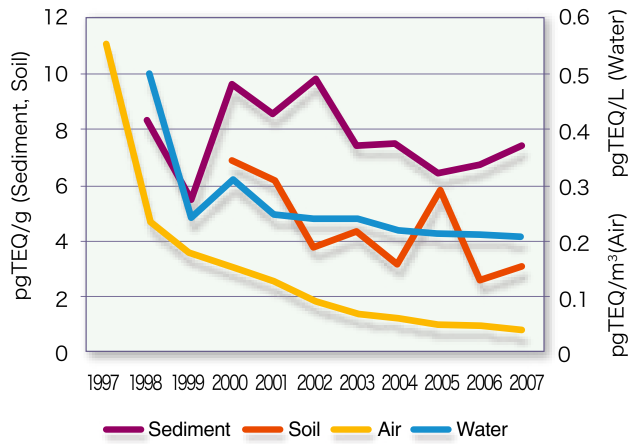



Figure 19 Temporal trends of environmental dioxin concentrations (average) in Japan

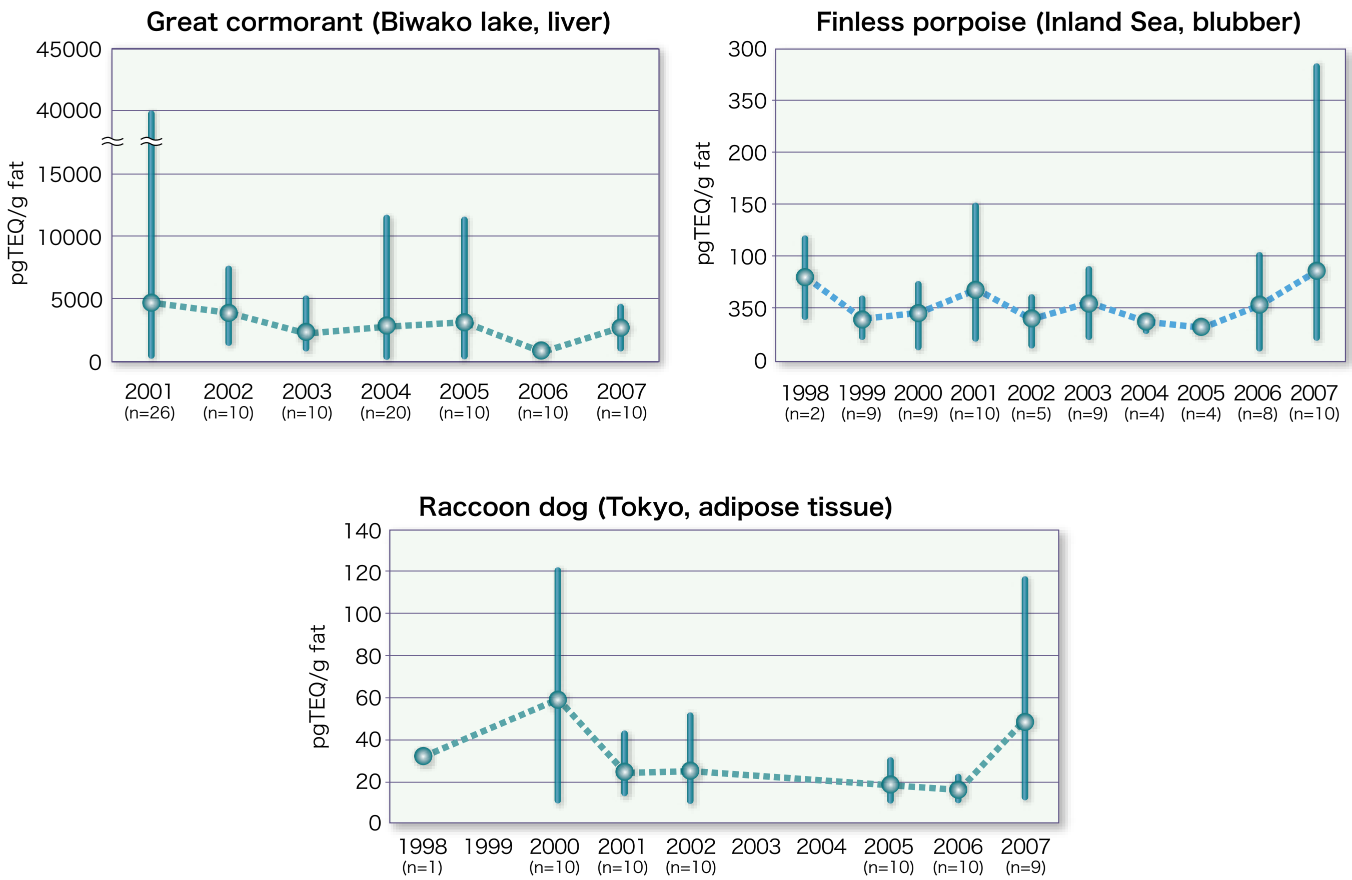



Figure 20 Temporal trends of dioxin concentrations in Japanese wildlife (maximum – average – minimum)

# 4. Discussion and Recommendations of the Committee

The dioxin concentrations found in the bodies of Japanese wildlife were not sufficiently high to provoke concern regarding the kind of serious impacts witnessed in some overseas cases. However, among higher order predators within the ecosystem, individuals with high concentrations have been found, and some eggs of great cormorants and large raptors in particular showed high enough concentrations to raise concern over abnormalities.

It has been suggested that adult female mammals and birds are likely to have lower dioxin concentrations than males owing to elimination of dioxins through birth, lactation and egg-laying, and the findings of this survey also showed such a tendency in some species. Findings also revealed differences in concentrations within the same individual according to organs and tissues, with liver in particular showing high accumulation in certain species. No decline was observed in dioxin concentrations in the bodies of wildlife in the same location over time.

Detailed analysis of the survey results will require the controlled setting of conditions such as age, sex, location in which specimens are collected, type of organ or tissue, year collected and so forth, and the collection of a sufficient number of individuals. Furthermore, considerations are required for the fact that the concentration of dioxins in wildlife is a result of accumulation for several years, and for the movements of animals. This survey did not gather enough specimens to enable this kind of precise investigation, but it did collect important data in an aspect of Japan's nature that had not been sufficiently investigated, shedding enough light on the dioxin concentrations in Japanese wildlife to enable comparison with overseas cases, and showing the differences that exist between different species in dioxin concentrations and types.

Histopathological investigation of impacts revealed changes likely to have been caused by dioxins in some specimens, but since no causal relationship with concentrations was found, the impacts of dioxin accumulation was unclear. However, evidence of impacts was found in certain physiological functions such as drug-metabolizing enzyme activity and thyroid hormone concentrations. It is not known at the present time whether such evidence will lead to concrete toxic impacts, but this evidence indicates that further investigation is required.

Impacts on immune functions and the central nervous system have been reported as effects of dioxin on wildlife, but methods of investigating such impacts in wildlife have yet to be established, and this survey was unable to investigate them. Concerning impacts on reproduction, it was not possible during this survey to conduct investigation of the relationship between dioxin concentrations in eggs and hatching ratios, and such areas need to be investigated in future.

This survey yielded important knowledge regarding the state of exposure of wildlife to dioxins, accumulation kinetics within the body, and changes over time. No declining trend in concentrations within the body indicates the need for examination of the effects of restriction of dioxin emissions through further investigation of specific species, age groups, sex, location, and types of organ or tissue. Elucidating the impacts of chemical substances on wildlife also requires systematic observation not only of exposure, but also of such factors as changes in wildlife numbers and presence of abnormalities in individuals. The accurate assessment of the risks to wildlife posed by chemical substances calls for integrated, systematic, long-term investigation, together with a consideration of coexistence with human beings.

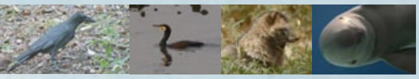
# Supplementary information

## 1. Toxic Equivalent (TEQ) and Toxic Equivalency Factor (TEF)

- ＊ Toxic Equivalency Factors (TEFs, Table 8), which are used to calculate Toxic Equivalents (TEQs), were proposed by the WHO in 1998 for humans and mammals, birds and fish owing to the differing susceptibilities of these classes of animals to dioxins. Because TEFs were based on the results of toxicity tests using laboratory animals, some researchers harbor doubts about their applicability in considering impacts on wildlife.
- ＊ In 2006, new TEFs were proposed for humans and mammals that mainly differed from previous factors in being set lower for mono-ortho Co-PCBs and higher for octachlorinated PCDDs and PCDFs, as a result of which the TEQs of species with a higher proportion of Co-PCBs became lower.
- ＊ To calculate the values for survey results shown in this brochure, 2006 TEFs were applied to all results, including birds and fish, for the sake of comparison.

Table 8 **Toxic Equivalency Factors (TEFs)**

| Compound | | WHO-2006 TEF | WHO-1998 TEF | | |
|---|---|---|---|---|---|
| | | | Human/ Mammals | Birds | Fish |
| PCDDs | 2,3,7,8-TCDD | 1 | 1 | 1 | 1 |
| | 1,2,3,7,8,-PeCDD | 1 | 1 | 1 | 1 |
| | 1,2,3,4,7,8-HxCDD | 0.1 | 0.1 | 0.05 | 0.5 |
| | 1,2,3,6,7,8-HxCDD | 0.1 | 0.1 | 0.01 | 0.01 |
| | 1,2,3,7,8,9-HxCDD | 0.1 | 0.1 | 0.1 | 0.01 |
| | 1,2,3,4,6,7,8-HpCDD | 0.01 | 0.01 | < 0.001 | 0.001 |
| | OCDD | 0.0003 | 0.0001 | 0.0001 | < 0.0001 |
| PCDFs | 2,3,7,8-TCDF | 0.1 | 0.1 | 1 | 0.05 |
| | 1,2,3,7,8-PeCDF | 0.03 | 0.05 | 0.1 | 0.05 |
| | 2,3,4,7,8-PeCDF | 0.3 | 0.5 | 1 | 0.5 |
| | 1,2,3,4,7,8-HxCDF | 0.1 | 0.1 | 0.1 | 0.1 |
| | 1,2,3,6,7,8-HxCDF | 0.1 | 0.1 | 0.1 | 0.1 |
| | 1,2,3,7,8,9-HxCDF | 0.1 | 0.1 | 0.1 | 0.1 |
| | 2,3,4,6,7,8-HxCDF | 0.1 | 0.1 | 0.1 | 0.1 |
| | 1,2,3,4,6,7,8-HpCDF | 0.01 | 0.01 | 0.01 | 0.01 |
| | 1,2,3,4,7,8,9-HpCDF | 0.01 | 0.01 | 0.01 | 0.01 |
| | OCDF | 0.0003 | 0.0001 | 0.0001 | < 0.0001 |
| Co-PCBs | 3,3',4,4'-TeCB (#77) | 0.0001 | 0.0001 | 0.05 | 0.0001 |
| *non-ortho* | 3,4,4',5-TeCB (#81) | 0.0003 | 0.0001 | 0.1 | 0.0005 |
| | 3,3',4,4',5-PeCB (#126) | 0.1 | 0.1 | 0.1 | 0.005 |
| | 3,3',4,4',5,5'-HxCB (#169) | 0.03 | 0.01 | 0.001 | 0.00005 |
| *mono-ortho* | 2,3,3',4,4'-PeCB (#105) | 0.00003 | 0.0001 | 0.0001 | < 0.000005 |
| | 2,3,4,4',5-PeCB (#114) | 0.00003 | 0.0005 | 0.0001 | < 0.000005 |
| | 2,3',4,4',5-PeCB (#118) | 0.00003 | 0.0001 | 0.00001 | < 0.000005 |
| | 2',3,4,4',5-PeCB (#123) | 0.00003 | 0.0001 | 0.00001 | < 0.000005 |
| | 2,3,3',4,4',5-HxCB (#156) | 0.00003 | 0.0005 | 0.0001 | < 0.000005 |
| | 2,3,3',4,4',5'-HxCB (#157) ' | 0.00003 | 0.0005 | 0.0001 | < 0.000005 |
| | 2,3',4,4',5,5'-HxCB (#167) | 0.00003 | 0.00001 | 0.00001 | < 0.000005 |
| | 2,3,3',4,4',5,5'-HpCB (#189) | 0.00003 | 0.0001 | < 0.00001 | < 0.000005 |





## 2. Treatment of results below the detection limit

＊ Three different methods for calculating averages and TEQs can be applied to the treatment of analysis results below the detection limit: (1) count as 0; (2) count as 1/2 of the value of detection limit; (3) count as the value of detection limit.

＊ Since it is inconceivable that absolutely no dioxin is present in the bodies of wild animals, the method most frequently used by the Ministry of the Environment is to count results below the detection limit as 1/2 of that value, and so the same method was used for this brochure. Results calculated using each of the three methods are shown in the report for each year.

＊ During the ten years that the survey on dioxins in Japanese wildlife has been conducted, analytical technologies have evolved and the detection limit has become lower. As a result, the value applied to the same "below the detection limit" has also become smaller with every passing year.

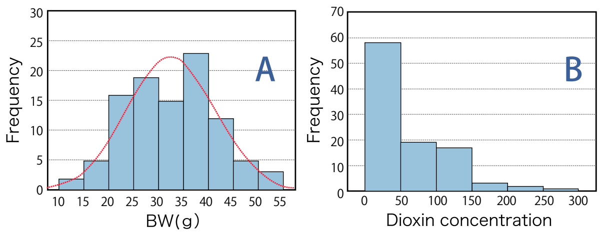
＊ Up to 2001, moreover, the quantitative limit was used rather than the detection limit, with 1/2 of the quantitative limit being used to represent results below the quantitative limit for calculating TEQs. Since the quantitative limit is about three times higher than the detection limit, TEQs for low concentrations were accordingly higher for years before 2001 than for 2001 onwards.

## 3. Statistical processing

＊ When comparing variables of two groups, statistical tests are conducted to determine whether the difference between the two groups is significant or not. In this brochure, if the probability that no difference exists is below 5% ($P<0.05$), the result is described as a statistically significant difference.

＊ Statistical tests are also used to determine whether a significant correlation exists between two variables. In this brochure, if the probability that no correlation exists is below 5% ($p<0.05$), the result is described as a statistically significant correlation. If one variable increases when the other increases, the correlation is described as positive, and if one variable increases when the other decreases, the correlation is described as negative. A calculated value called the correlation coefficient (r) is used to indicate the degree of correlation on a scale of 0 to 1, with $r = 1$ representing the strongest correlation.

＊ Most cases of statistical processing use normally distributed variables. For example, Fig. A on the right shows a normal bell-shaped distribution of the body weights of large Japanese field mice collected in Saitama Prefecture for this survey. However, as Fig. B shows, dioxin concentrations found in the bodies of the same animals were not distributed normally.




＊ Nonparametric statistical methods are used to analyze such variables that do not show normal distributions.

＊ The nonparametric methods used for this brochure are the Mann-Whitney U-test to compare results of analysis, and Spearman's rank correlation to examine correlations.

＊ The Mann-Whitney U-test is a nonparametric method that can be used in place of the t-test for two independent samples. It assumes that the variables concerned are expressed by ranking scale. The interpretation of this test is basically identical to that of the result of a parametric two-sample t-test except when calculations are based on rank sum rather than average.

＊ The correlation coefficient for Spearman's rank correlation can be thought of as a standard Pearson's product-moment correlation coefficient, or in other words, in terms of the ratio of the distribution to be explained, except when calculated from rank.

## 4. Adverse effects

∗ To investigate impacts under this survey, liver drug-metabolizing enzyme activity and concentrations of thyroid and sex steroid hormones in the blood were measured, and thyroid glands and major organs were histopathologically examined. This is based on the previous findings on impacts in specimens accidentally exposed to high dioxin concentrations, wildlife from locations with high dioxin concentrations, and laboratory animals experimentally exposed to dioxins.

∗ When dioxins enter the body, they bind with receptors (AHR) in cytoplasm and translocate to cell nuclei, where they bind with a different protein (ARNT) and bind to the specific sequences in genes responsible for producing drug-metabolizing enzymes. This promotes gene transcription, inducing enzyme activity. As a result, physiological function is potentially disrupted and the situation such as hormone imbalance may develop. The extent to which this reaction is occurring can be estimated by measuring drug-metabolizing enzyme activity.

∗ Blood thyroid hormone concentrations have been reported to decline on exposure to dioxins. This is thought to be the result of such mechanisms as dioxins binding with receptors (AHR) to induce enzymes that conjugate thyroid hormones, causing them to be excreted, and the competitive binding of certain dioxin metabolites with one of blood proteins that normally binds with thyroid hormones.

∗ Regarding sex steroid hormones, blood concentrations have been reported to decline as a result of inhibition of their synthesis by dioxins, while estradiol concentrations have been reported to rise.

∗ Dioxins have been shown to cause cancer in various tissues including liver and thyroid, and hyperplasia of liver and thyroid gland has also been reported.

∗ Changes such as these that occur in the body as a result of exposure to dioxins could be used as indicators of the extent of impacts on wildlife.

∗ This survey indicated the possibility of using drug-metabolizing enzyme activity and thyroid hormone concentrations as indicators of the impact of dioxin accumulation in great cormorants, jungle crows, and large Japanese field mice.

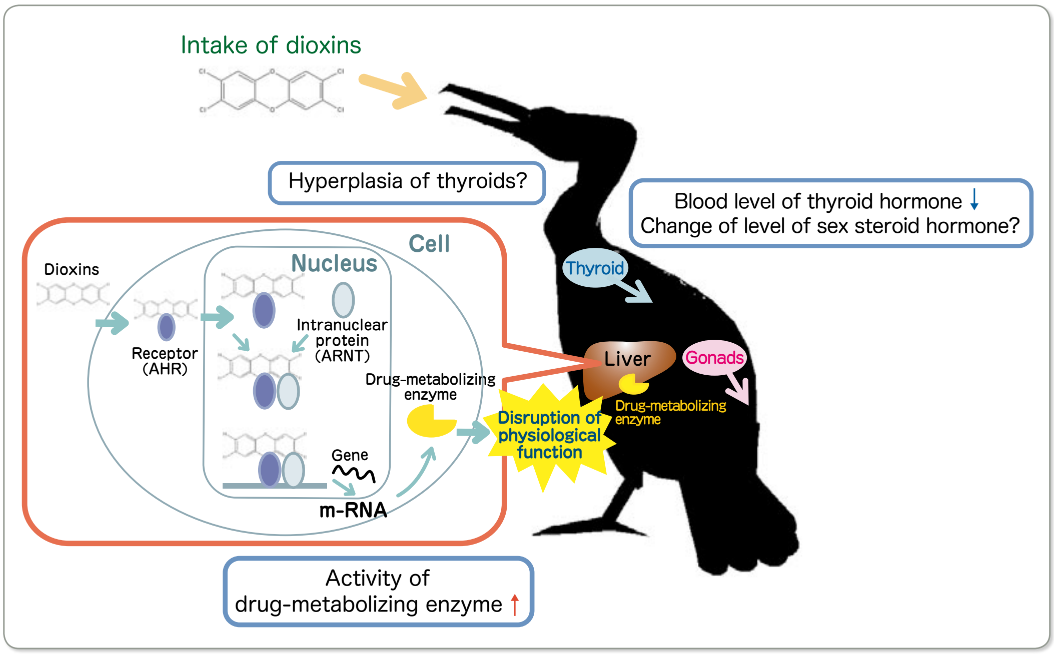



Figure 21 Impacts of dioxins on animals

# References

Survey reports used to create this brochure can be viewed on the Ministry of the Environment website through the following links in Japanese:


- Survey on the State of Dioxin Accumulation in Wildlife
  http://www.env.go.jp/chemi/dioxin/chosa/yasei.html

- Survey on the State of Impacts of Endocrine Disruptors on Wildlife
  http://www.env.go.jp/chemi/end/kento1302/index.html (Document 4)
  http://www.env.go.jp/chemi/end/kento1402/index.html (Document 2-4)
  http://www.env.go.jp/chemi/end/kento1502/index.html (Document 2-1-3, 2-2)
  http://www.env.go.jp/chemi/end/kento1602/index.html (Document 2-1-3, 2-2)
  http://www.env.go.jp/chemi/kurohon/2005/http2005/30furoku/311.pdf


## Other reports


1 ) Ministry of the Environment website listing survey results and reports related to dioxin countermeasures
Dioxin Emission Inventory
Results of environmental surveys on dioxins
http://www.env.go.jp/chemi/dioxin/report.html

2 ) Ministry of Health, Labour and Welfare website on dioxin countermeasures including survey reports of daily dietary intake of dioxins
http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/dioxin/index.html

3 ) Fisheries Agency website of surveys on the state of dioxin accumulation in seafood
http://www.maff.go.jp/j/syouan/tikusui/gyokai/g_kenko/busitu/index.html#dai

4 ) Fukushima Prefecture website of report of results of a survey on exogenous endocrine disruptors in wildlife (including dioxins)
http://www.pref.fukushima.jp/kankyou/taiki/daiokisin_top.html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1 ) Kumar, K.S. et al. (2002) Distribution and elimination of polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, and p,p'-DDE in tissues of bald eagles from the upper peninsula of Michigan. Environ. Sci. Technol. 36(13):2789-2796.

2 ) Yamashita, N. et al. (1993) Embryonic abnormalities and organochlorine contamination in double-crested cormorants and Caspian terns from the upper Great Lakes in 1988. Environmental Pollution. 70:163-173.

3 ) Ministry of the Environment (2008) The Accumulation of Dioxins in the Japanese People.
http://www.env.go.jp/chemi/dioxin/pamph/cd/index.html

4 ) Ministry of Health, Labour and Welfare (2000-2007) Surveys of daily dietary dioxin intake (in Japanese) (data compiled from FY1999-2006 reports);
http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/dioxin/index.html

5 ) Ministry of the Environment (2003) FY 2002 Report of the Golden Eagle Conservation Program (in Japanese).

6 ) Elliott JE et al. (1996) Biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in bald eagle (Haliaeetus leucocephalus) chicks. Environmental Toxicology and Chemistry 15:782-793.

7 ) Hilscherova K. et al. (2003) Oxidative stress in laboratory-incubated double-crested cormorant eggs collected from the Great lakes. Arch. Environ. Contam. Toxicol. 45:533-546.

8 ) Ministry of the Environment (2008) Dioxin Emission Inventory (in Japanese).
http://www.env.go.jp/air/report/h20-08/full.pdf

9 ) Ministry of the Environment (2008) Environmental Survey of Dioxins in FY 2007 (in Japanese).
http://www.env.go.jp/air/report/h20-06/full.pdf

## Reference books


1 ) Satoru Suzuki (Ed.): Molecular Approaches to Environmental Pollution. Tokai University Press, 253pp. (2009) (in Japanese).
2 ) Chisato Mori and Emiko Todaka.: Inner Space Pollution - A warning from umbilical cord to protect future generations (Shiritai Science series), Gijutsu-Hyohron CO., Ltd. 208pp. (2008) (in Japanese).
3 ) Masatoshi Morita and Hirohisa Takano.: Environmental Science series Iwanami Shoten No.8, Health and the Environment, 232pp. (2005) (in Japanese).
4 ) Tohru Inoue and Taisen Iguchi (Ed.): Endocrine Disruptor in High-Integrated Biosystem, Springer-Verlag, Tokyo, 321pp. (2005) (in Japanese).


## For further information on dioxins and wildlife:

### Technical books


1 ) Arnold Schecter ed. (1994) Dioxins and Health. Plenum Press, New York and London.
2 ) Interdisciplinary Studies on Environmental Chemistry, Murakami, Vol. 1, Biological Responses to Chemical Pollutants, Y., Nakayama, K., Kitamura, S., Iwata, H. and Tanabe, S. (Eds), TERRAPUB, Tokyo, Japan, 372 pp. (2008)


### Review papers


1 ) Tanabe, S. and Kunisue, T. (2005): Contamination by dioxins and related compounds in wildlife and human. The Japanese Journal of Toxicology, 18, 319-331(in Japanese).
2 ) Shinsuke Tanabe (2005): Global Pollution, Biological Accumulation and Adverse Effects of Toxic Environmental Contaminants. Environmental Science, 18(2), 191-198 (in Japanese).
3 ) Tanabe, S., Kunisue, T. and Takahashi, S. (2006): Contamination by persistent toxic substances and their safety for human breast milk in the Asian region. Iden (Genetics-heredity), Special issue No.19, 104-115 (in Japanese).


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(Titles omitted. Names presented in Japanese syllabic order by family name. Affiliations as of March 2009.)

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| Yasuyuki Ohshima[2] | Professor emeritus, Waseda University |
| Fumio Sugimori[3] | Director, Abiko City Museum of Birds |
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| Toshio Tsubota | Professor, Graduate School of Veterinary Medicine, Hokkaido University |
| Shin-ichi Hayama | Associate professor, Faculty of Veterinary Science, Nippon Veterinary and Life Science University |
| Yoshihiro Fujise | Director, Research Department, The Institute of Cetacean Research |
| Yasuo Minagawa | Director, Wildlife Volunteer Center |
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We would like to express our heartfelt gratitude to local governments and hunting clubs of survey areas, and all other organizations and individuals who cooperated in the implementation of this survey.

2009

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