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 *non-ortho*	3,3',4,4'-TeCB（#77）	0.0001	0.0001	0.05	0.0001
	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.

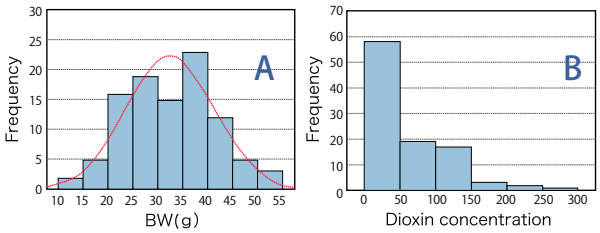
* Up to 2001, moreover, the quantitative limit was used rather than the detection limit, with 1/2 of the quantitative limit being used to represent results below the quantitative limit for calculating TEQs. Since the quantitative limit is about three times higher than the detection limit, TEQs for low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

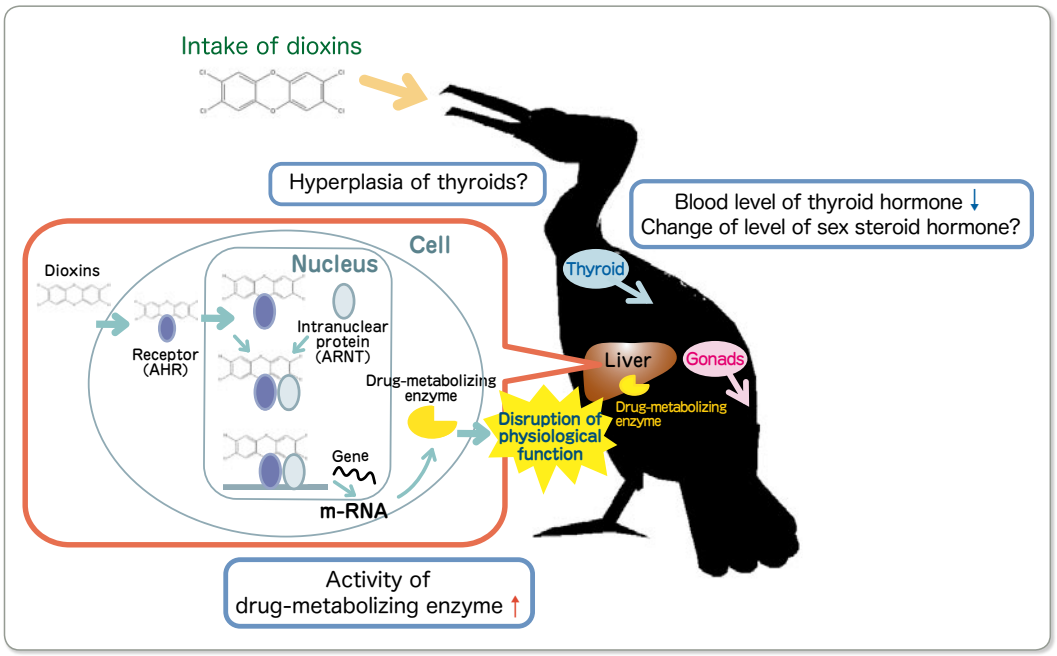


Figure 21 Impacts of dioxins on animals

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

Bibliography

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).

Members of the Committee for the Survey on the State of Dioxins Accumulation in Wildlife

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

Taisen Iguchi	Professor, Center for Integrative Bioscience, Okazaki National Research Institutes
Gen Ohi[1)]	Professor emeritus, the University of Tokyo
Yasuyuki Ohshima[2)]	Professor emeritus, Waseda University
Fumio Sugimori[3)]	Director, Abiko City Museum of Birds
Shinsuke Tanabe	Professor, Center for Marine Environment Studies, Ehime University
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
Chisato Mori	Professor, Graduate School of Medicine, Chiba University
Masayuki Yasuno[3)]	Retired professor, the University of Shiga Prefecture
Tadasu Yamada	Head, Division of Vertebrates, Department of Zoology, The National Museum of Nature and Science

1) Chair
2) Deceased
3) Until FY2004

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

Please address opinions and inquiries to:

Environmental Risk Assessment Office

Environmental Health Department
Ministry of the Environment, Japan

TEL : +81-3-3581-3351 (extension 6343)
FAX : +81-3-3581-3578

http://www.env.go.jp/