

Report on the Test Results of Endocrine Disrupting Effects of Tributyltin (TBT) on Fish (Draft)

1. Background and development

In its SPEED (Strategic Programs on Environmental Endocrine Disrupters) '98¹⁾ published in May, 1998, Japanese Environment Agency (Ministry of the Environment, MoE, from 2001) listed 67 substances suspected to have endocrine disrupting effects. After that, the Agency carried out national environmental surveys, and decided to "set to risk assessment, while hearing expert opinions, to start with the four substances(i.e. Tributyltin, Nonyl phenol, 4-Octylphenol, and Di-*n*-butyl phthalate.) classified as priority substances for risk assessment, etc., based on the environmental survey and literature search results" at the Meeting of the Advisory Committee on Endocrine Disrupters (chairman: Dr. Tsuguyoshi Suzuki, professor emeritus of the University of Tokyo) held in October, 1999.

Further, in the Prime Minister's Decision in December, 1999, it was decided to carry out risk assessments on more than 40 substances suspected to have endocrine disrupting effects in three years starting from FY 2000, as part of the Government's Millennium Project. At the meetings of the Advisory Committee on Endocrine Disrupters held in July and October 2000, and in March 2001, studies were made on the screening and test methods on the human health effects and ecological effects of the 12 substances, including the above-mentioned four priority chemicals selected to be surveyed preferentially in FY 2000, and successively tests and researches have been conducted.

As for tributyltin (TBT) compounds, they were selected in the list of priority substances, based on the report that they were suspected of having endocrine disrupting effects since imposex was observed in aquatic organisms other than fish, especially in rock shell, where TBT compounds were found in higher concentration than their ordinary concentration in the water environment. Efforts have been made by MoE to confirm whether or not and how much TBT has endocrine disrupting effects, centering on mammals and fish for which the development of test methods is now in progress at the OECD level. This draft report describes the TBT's endocrine disrupting effects, etc. on fish, including vitellogenin assay and partial life cycle test using fish (Japanese medaka, killifish), as well as the results, etc. obtained so far in the literature search and reliability assessment.

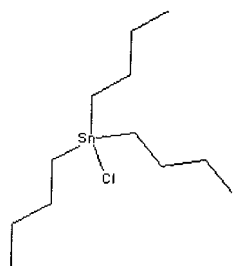
As for TBT's effects on human health, MoE is now conducting tests using rodents for assessing toxicity, and intending to report the results of those tests together with the exposure assessment results obtained from the planned diet surveys, etc.

Though there are homologues such as bis-tributyltin oxide, tributyltin chloride, etc. among tributyltin compounds, the tests were made using tributyltin chloride made by Wako Pure Chemical Industries, Ltd., since tributyltin chloride was found in the literature search, etc. to show the lowest observed-effect concentration in the water suspected of having endocrine disrupting effects. As individual tributyltin compounds cannot be identified separately in the environment, free tributyltin was measured in the environmental survey, and its values were converted into tributyltin chloride for comparison.

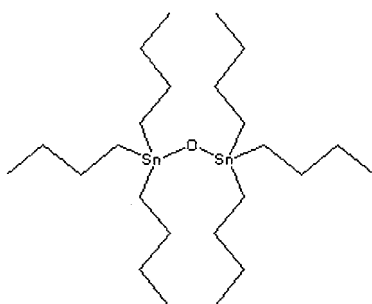
2. Properties, uses, etc.

(1) Chemical structure

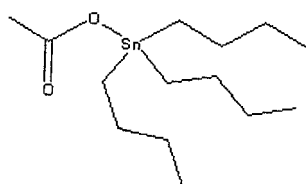
Structural formulas of major tributyltin compounds are shown as follows:



Tributyltin chloride



bis-Tributyltin oxide



Tributyltin acetate

Fig.1 Structural formulas of major tributyltin compounds

(2) Major properties^{N.B.)}

Major properties of tributyltin compounds as reported in the literature information obtained through JICST and in the World Health Organization (WHO) Environmental Health Criteria are summarized as follows²⁾. A reliability assessment has not been made by MoE.

Table 1 Major properties of tributyltin compounds

	Tributyltin chloride (TBTC)	bis-Tributyltin oxide (BTBO)	Tributyltin acetate (TBTAc)
Cas No.	1461-22-9	56-35-9	56-36-0
Molecular formula	C ₁₂ H ₂₇ ClSn	C ₂₄ H ₅₀ O ₂ Sn ₂	C ₁₈ H ₃₅ O ₂ Sn
Molecular weight	325.49g/mole	596.07g/mole	349.08 g/mole
Appearance	Colorless, transparent liquid; peculiar irritating odor	Colorless or slightly yellowish, transparent liquid; peculiar irritating odor	White powder
Specific gravity	1.2(20 °C)	1.17-1.18	1.4942(20 °C)
Melting point	-16	<-45	84.5-85
Vapor pressure	0.00927mmHg(25 °C)	0.0016mmHg(25 °C)	
Water solubility	0.74786mg/L(25 °C)	0.75mg/L	
Log K _{ow}	4.76	3.19-3.84	3.24

N.B.: Compiled from World Health Organization (1990) Tributyltin compounds, Environmental Health Criteria 116.

Note: MoE has not made specific reliability assessment on the literature listed in WHO's Environmental Health Criteria. (The same applies in the following.)

(3) Uses

The reports on the uses of tributyltin compounds covered in the literature information obtained through JICST, etc. and in WHO's Environmental Health Criteria are described below:

- Tributyltin compounds were used as germicides for pears and onions; mollusk eliminators; rodent repellants and insecticides for wood, textile, paper, leather and glass products; antifouling paints for fishing nets, ship bottom and neoprene rubber; industrial germicides for paper, wood, paint, leather and textile working; reducing agents; catalyst for fireproof polyester, hardening agent, waterproofing, antioxidant and corrosion inhibitor; and for medical purposes^{3,4}.
- According to the report in WHO's Environmental Health Criteria², tributyltin compounds were used as mollusk expellants against freshwater snails, intermediate host for Trematoda parasites (vector of schistosomiasis); antifouling paints for boats, ships, quays, bouys, crab baskets, fishing nets, etc.; wood preservatives; sliming agents for stone work; and germicides and biocides for refrigerating systems, power plant cooling towers, paper & pulp plants, breweries, leather works, textile mills, etc.

3. State of use, regulation, etc.

(1) State of use and regulation in Japan

- In Japan, tributyltin compounds (tributyltin oxide) were invalidated as agricultural chemicals in 1977, and their use in household products was forbidden in 1979. Thereafter, as the state of environmental pollution became evident, their use in fish farm nets was banned voluntarily by the National Federation of Fisheries Cooperatives in 1987. In 1989, tributyltin oxide was designated as a Class I Specified Chemical Substance under the Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances Class I, and the manufacture, import and use of this chemical were prohibited in principle. Further, in 1990, 13 kinds of compounds including tributyltin chloride, were designated as Class II Specified Chemical Compounds, and became subject to the regulation on their use, etc. Following this, the shipbuilding industry, led by the Shipbuilders Association of Japan, stopped using it, and the Japan Paint Manufacturers Association voluntarily reduced its use. The use of TBT proper as raw material of antifouling paints was 11,840 ton in 1989, but the production of TBT-containing paints has been stopped since 1997⁵.
- Acceptable daily intake of bis-tributyltin oxide is set at 1.6 [g/kg/day in Japan⁶].

(2) State of production and regulation outside Japan⁵

- World production of organic tin compounds was estimated at about 50,000 ton per year in 1996, of which the production of TBT proper was estimated at several thousand ton per year. It is said that the world total consumption of TBT-containing paints has little changed.
- The state of regulation outside Japan is given in Appendix 1.

4. Distribution and degradation in environment, fate in body, and concentration in the environment

(1) Distribution and degradation in the environment

(a) Distribution in the environment

Fig.2 shows the measurement results of tributyltin (TBT) concentration in the environment conducted in Lake Westeinder in the Netherlands. TBT concentration in the water was undetected (<

20ng/L). Comparing average concentrations in the bodies of organisms, they were 610 [g/kg in zoo-plankton, 3,091 [g/kg in filter-feeding shellfish (mussels), 886 [g/kg in animal-feeding small fish (such as bream and tench of Cyprinidae, smelt of Osmeridae), 442 [g/kg in animal-feeding large fish (such as pike of Esocidae), 288 [g/kg in eels, and 27 [g/kg in fish-feeding aquatic birds (cormorant). Such a trend as the higher the trophic level, the higher the concentration in the body was not observed⁷⁾.

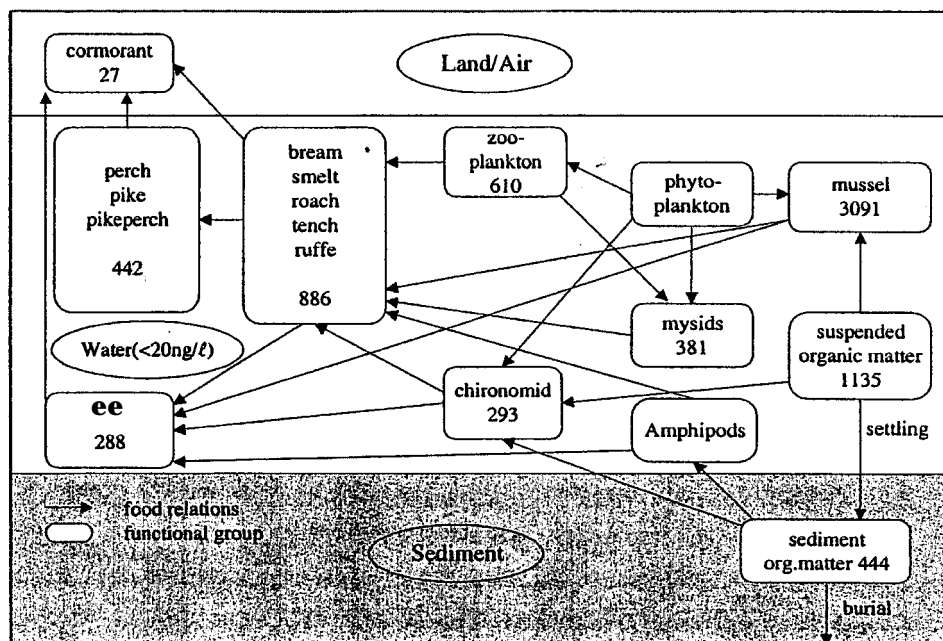


Fig. 2 Concentration of TBT in aquatic organisms on the food webs in the lake & marsh ecology (Calculated from Stab, J. A. et al. (1996)⁷⁾)

(b) Degradability

The reports on the degradability of tributyltin compounds covered in the WHO's Environmental Health Criteria²⁾ are summarized below.

- Slesinger and Dresser⁸⁾ reported biodegradation of tributyltin oxide by microorganisms separated from activated sludge and soil. As a result, half-life was 70 days in aerobic conditions, and 200 days in anaerobic conditions.
- Thain et al.⁹⁾ suggested half-life of tributyltin oxide was 6 days in fresh water, and 60-90 days in sea water at 5 °C.
- Seligman et al.¹⁰⁾ reported half-life of tributyltin oxide was 5-9 days in brackish water at 28 °C, and 7-11 days in sea water at 12 °C.
- Maguire et al.¹¹⁾ described the characteristics of half-time of tributyltin oxide as follows:
 -] Loss of tributyltin oxide due to volatilization is much limited in degradation process, because its half-life was longer than 11 months.
 -] Half-life of tributyltin oxide by hydrolysis is as slow as 11 months, too.
 -] The importance of photolysis of tributyltin oxide is higher, as compared with that of volatilization and hydrolysis, but its half-life by photolysis is longer than 3 months. This degradation process is possible theoretically, but not effective in the turbid or colored environmental water with insufficient transmission of ultraviolet rays.
 -] Aerobic biodegradation occurs in water and sediment. Its half-life varies considerably depending conditions, ranging from 4 to 5 months.

] Anaerobic biodegradation occurs in water and sediment. Its half-life varies considerably, around one and a half months.

(2) Fate in the body

The reports on the internal fate (accumulation and excretion) of tributyltin compounds in WHO's Environmental Health Criteria²⁾ are summarized below.

Table 2 Internal fate (accumulation and excretion) of tributyltin compounds

	Specific name	Biological accumulation factor	Author
Phyto-plankton	Green algae <i>Ankistrodesmus falcatus</i>	30,000 (TBTO:20 [g/L])	Maguire <i>et al.</i> (1984) ¹²⁾
	Haptophyceae <i>Isochrysis galbano</i>	5,500	Laughlin <i>et al.</i> (1986) ¹³⁾
Mollusc	Japanese oyster <i>Crassostrea gigas</i>	6,000(TBTO:0.15 [g/L]) 2,000(TBTO:1.25 [g/L])	Waldock <i>et al.</i> (1983) ¹⁴⁾
	European oyster <i>Ostrea edulis</i>	1,500(TBTO:0.15 [g/L]) 1,000(TBTO:1.25 [g/L])	
	Blue mussel <i>Mytilus edulis</i>	1,000-17,000 (TBTO:0.023-10.67 [g/L])	Laughlin <i>et al.</i> (1986) ¹³⁾
	Blue mussel <i>Mytilus edulis</i>	5,000-160,000	Cheng and Jensen(1989) ¹⁵⁾
	Gyraulid <i>Biomphalaria glabrata</i>	<50	Allen <i>et al.</i> (1980) ¹⁶⁾
Arthropoda	Crabs <i>Rhithropanopeus harrisi</i>	60 (TBTO:0.28 [g/L])	Allen <i>et al.</i> (1980) ¹⁶⁾
Fish	Sheephead minnow <i>Cyprinodon variegatus</i>	2,600 (TBTO:0.96-2.07 [g/L])	Ward <i>et al.</i> (1981) ¹⁷⁾
	Mullets <i>Liza aurata</i>	20-30 (liver, kidney) (TBTO: [g/L])	Bressa <i>et al.</i> (1984) ¹⁸⁾
	Chinook salmon <i>Oncorhynchus tshawytscha</i>	4,300 (liver) 1,300 (brain) 200 (muscle) (TBTO:1.49 [g/L])	Short and Thrower (1986) ¹⁹⁾
	Carp <i>Cyprinus carpio</i>	1,000(TBTO:0.0018 ±0.0024 [g/L])	Tsuda <i>et al.</i> (1987) ²⁰⁾
	Crucian carp <i>Carassius carassius grandoculis</i>	360-13,400 (TBTO)	Tsuda <i>et al.</i> (1986) ²¹⁾

	Specific name	Excretion speed	Author
Mollusc	Blue mussel <i>Mytilus edulis</i>	Half-life Organic tin: 40 days Total tin: 25 days	Cheng and Jensen(1989) ¹⁵⁾
Fish	Sheepshead minnow <i>Cyprinodon variegatus</i>	Excretion rate after 20 days Muscle: 74 % Internal organs: 80 %	Ward <i>et al.</i> (1981) ¹⁷⁾

(3) Result of environmental surveys in Japan

(a) Nationwide Exogenous Endocrine Disrupters survey, etc.

The concentration of tributyltin in the water, bottom sediment, soil, aquatic organism and wildlife at a total of 1,373 samples all over the country was measured in the Nationwide Urgent Exogenous Endocrine Disrupters Survey (1998) and the Nationwide Exogenous Endocrine Disrupters Survey (1999) conducted by the Environment Agency, as well as in the Environmental Endocrine Disrupters Survey at Public Water Areas (1998) and the Environmental Endocrine Disrupters Survey at Public Water Areas (1999) conducted by the Ministry of Construction. Here the measured results are shown in concentration of tributyltin chloride. According to the results, in the water quality survey, tributyltin chloride was detected at 52 samples out of a total of 598 samples in two years (detection ratio: 8.7 %), and its concentration range was NDs (0.001 - 2.2) - 0.098 [g/L. The arithmetic mean concentration was 0.00047 [g/L (assuming NDs = 0), and 95th percentile concentration^{N.B.)} was 0.003 [g/L. The arithmetic mean concentration was 0.0097 [g/L and 0.019 [g/L if calculated assuming that NDs are half of the detection limits and equal to them, respectively. Concentration at median, 75th percentile^{N.B.)} and 90th percentile^{N.B.)} was NDs (detection limits: 0.01 [g/L).

In the sea area alone, tributyltin chloride was detected at 33 samples of total 81 samples in two years (detection ratio: 41 %), and its concentration range was NDs (0.002 - 0.01) - 0.098 [g/L. The arithmetic mean concentration was 0.0027 [g/L (assuming NDs = 0), and median, 75th percentile, 90th percentile and 95th percentile concentrations were ND, 0.003 [g/L, 0.004 [g/L and 0.007 [g/L, respectively. But the arithmetic mean concentration was 0.0042 [g/L and 0.0057 [g/L if calculated assuming that NDs are half of the detection limits and equal to them, respectively.

In the areas except the sea area, tributyltin chloride was detected at 19 samples out of total 517 samples in two years (detection ratio: 3.7 %), and its concentration range was NDs (0.001 - 2.2) - 0.0066 [g/L. The arithmetic mean concentration was 0.00012 [g/L (assuming NDs = 0), and concentration at median, 75th, 90th and 95th percentile was NDs (0.01 [g/L). But the arithmetic mean concentration was 0.011 [g/L and 0.021 [g/L if calculated assuming that NDs are half of the detection limits and equal to them, respectively.

In the bottom sediment survey, tributyltin chloride was detected at 130 samples out of total 242 samples in two years (detection ratio: 54 %), and its concentration range was NDs (0.1 - 22) - 218 [g/kg. The arithmetic mean concentration was 8.0 [g/kg (assuming NDs = 0), and median, 75th percentile, 90th percentile and 95th percentile concentrations were 0.2 [g/kg, 1.2 [g/kg, 13.1 [g/kg and 57.9 [g/kg, respectively. But the arithmetic mean concentration was 8.5 [g/kg and 9.1 [g/kg if calculated assuming that NDs are half of the detection limits and equal to them, respectively.

In the aquatic organism survey, tributyltin chloride was detected at 113 samples out of total 141 samples (detection ratio: 80 %). Its concentration range was NDs (1.1) - 131 [g/kg, and arithmetic mean concentration was 12.3 [g/kg (assuming NDs = 0). Median, 75th percentile, 90th percentile and 95th percentile concentrations were 3.3 [g/kg, 7.6 [g/kg, 40.4 [g/kg and 86.2 [g/kg, respectively. But the arithmetic mean concentration was 12.4 [g/kg and 12.5 [g/kg if calculated assuming that NDs are half of the detection limits and equal to them, respectively.

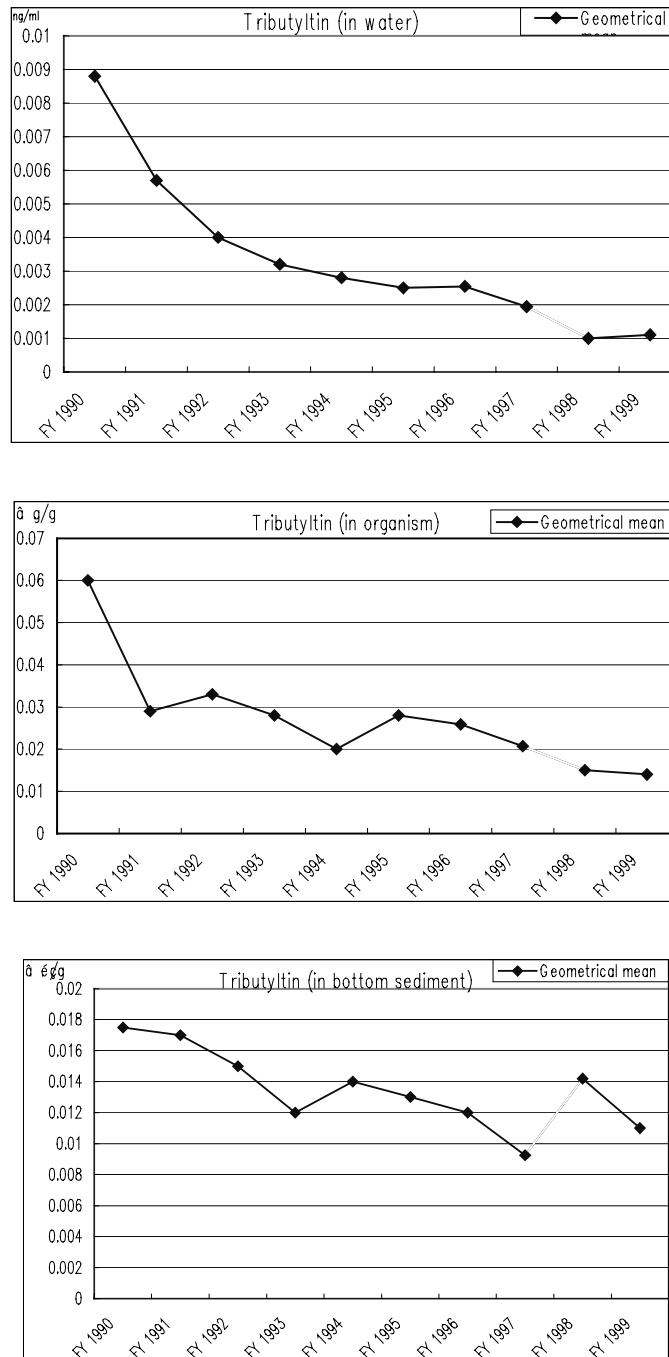
N.B.: 75th percentile: The 3/4th value of all the measured values when they are placed in the ascending order.

90th percentile: The 9/10th value of all the measured values when they are placed in the ascending order.

95th percentile: The 95/100th value of all the measured values when they are placed in the ascending order.

(b) Results on environmental surveys in Japan

According to the environmental surveys conducted by Japan Environment Agency in the period between FY 1990 to FY 1999, tributyltin compounds remained widely in the environment. Their pollution level was on the tendency of improving in organism and water, but showed hardly any marked change in bottom sediment.



From FY 1998 on, detection sensitivity is improved by the modification of analysis methods.

Fig. 3 Secular change in tributyltin concentration (N.B.: in terms of tributyltin chloride concentration)

In its evaluation of the survey results (1999), etc., Japan Environment Agency said: "Given the current state of production * of tributyltin compounds in Japan, their environmental pollution is expected to improve further. Considering possible pollution due to existence of some states and regions having no control on tributyltin compounds yet, however, it is necessary to continue countermeasures against, and monitoring the state of, environmental pollution caused by such compounds. In view of the suggestions that tributyltin may have endocrine disrupting effects, it is also necessary to accumulate knowledge on its toxicity, including related information."

*: The fact that there is practically no production/use of tributyltin compounds for open-end application in Japan.

5. General toxicity

(1) Acute toxicity

(a) Fish

Mentioned below are the summary of the reports on acute toxicity to fish covered in the WHO Environmental Health Criteria²⁾.

- Acute toxicity tests, using chinook salmon, soles, greenfish, longchin goby, sticklebacks, minnows and carps, poachers, Japanese medaka (killifish), guppy, common carp, and bluegill as test organisms, are reported by Short and Thrower²²⁾, Thain²³⁾, Kakuno and Kimura²⁴⁾, Shimizu and Kimura²⁵⁾, RIVM²⁶⁾, Linden et al.²⁷⁾, Temmink and Everts²⁸⁾, and Plum²⁹⁾. As a result, 96-hour median lethal concentration LC₅₀ was in the range of 1.6-262 [g/L (in terms of tributyltin chloride), and the lowest LC₅₀ value was observed for chinook salmon (*Oncorhynchus tshawytscha*)²²⁾.

(b) Aquatic invertebrates

The reports on acute toxicity to aquatic invertebrates except fish covered in the WHO Environmental Health Criteria²⁾ are summarized below.

- Acute toxicity tests, using mysids, copepoda, crangonid shrimps, *Lismata amboinensis*, *Daphnia*, sand crab, *Palaemon paucidens*, pond-snail, mussel, European oyster and common oyster as test organisms, are reported by Goodman et al.³⁰⁾, U'ren³¹⁾, Thain²³⁾, Linden et al.²⁷⁾, Meador³²⁾, Walsh³³⁾, and Temmink and Everts²⁸⁾. As a result, 96-hour LC₅₀ was in the range of 1.1-317 [g/L (in terms of tributyltin chloride), and the lowest LC₅₀ value was observed for mysids (*Mysidopsis bahia*)³⁰⁾.

(c) Aquatic algae and plants

The reports on acute toxicity to aquatic algae and plants covered in the WHO Environmental Health Criteria²⁾ are summarized below.

- Acute toxicity tests, using phytoplankton and link confetti, duckweed, and Potamogetonaceae as test organisms, are reported by Davis et al.³⁴⁾, Walsh et al.³⁵⁾, Thain²³⁾, Salazar³⁶⁾, Wong et al.³⁷⁾, Beaumont and Newman³⁸⁾, RIVM²⁶⁾, Floch et al.³⁹⁾, Deschiens and Floch⁴⁰⁾, and Bokranz and Plum⁴¹⁾. As a result, effects were observed in the range of 0.001-1,092 [g/L (in terms of tributyltin chloride), and EC₅₀ was in the range of 0.001-70 [g/L. The effect observed in the lowest 0.001 [g/L was an impediment to adhesion of spores of link confetti (*Enteromorpha intestinalis*)³⁴⁾.

(2) Chronic toxicity

(a) Fish

The reports on chronic toxicity to fish covered in the WHO Environmental Health Criteria²⁾ are stated below.

- Effects on yoke sac larval rainbow trouts exposed to tributyltin chloride at 0.2, 1 or 5 [g/L for 110 days were studied by Seinen et al.⁴²⁾ As a result, all the trouts exposed to 5 [g/L tributyltin chloride (TBTCI) died within 12 days, but no death was observed in the group of trouts exposed to TBTCI at 1 [g/L or lower. In the group exposed to TBTCI at 0.2 [g/L or over, delay in growth depending on dose and increase in liver weight relative to body weight were observed.
- Effects on medaka exposed to tributyltin oxide for one month were studied by Wester et al.⁴³⁾ As a result, in the group of medakas exposed to 3.2 [g/L tributyltin oxide, increase in death rate, delay in growth, abnormal behavior, and vacuole formation in retinal epithelia were observed. No-Observed-Effect-Concentration for the whole body toxicity was reported to be 0.32 [g/L (0.35 [g/L in terms of TBTCI), and that for liver 1 [g/L (1.1 [g/L in terms of TBTCI).
- Effects on longchin goby exposed to tributyltin oxide for 12 weeks were studied by Shimizu and Kimura²⁵⁾. As a result, in the group of gobies exposed to tributyltin oxide at 2.1 [g/L (2.3 [g/L in terms of TBTCI), male maturity index fell significantly, but no effect on female maturity index and ovarian tissue was observed.
- Effects on embryos of medaka exposed to 3-30 [g/L tributyltin oxide were studied by Weis et al.⁴⁴⁾ As a result, some cases of death were observed in the group of medakas exposed to tributyltin oxide at 30 [g/L (33 [g/L in terms of TBTCI).

(b) Aquatic invertebrates

The reports on chronic toxicity to aquatic invertebrates except fish covered in the WHO Environmental Health Criteria²⁾ are summarized below.

- Effects on common oyster larvae exposed to 0.02-100 [g/L tributyltin acetate were studied by His and Robert⁴⁵⁾. As a result, in the group of larvae exposed to tributyltin acetate at 0.05 [g/L (0.05 [g/L in terms of TBTCI) or over, growth was inhibited and deaths were observed within 10 days. Non-Observed-Effect-Concentration on growth was reported to be 0.02 [g/L (0.02 [g/L in terms of TBTCI).
- Effects on blue mussel larvae exposed to tributyltin oxide at 0.1, 1.0 or 10.0 [g/L were studied by Beaumont and Budd⁴⁶⁾. As a result, the group of mussels exposed to 10 [g/L (11 [g/L in terms of TBTCI) died within five days, and the group exposed to 1.0 [g/L (1.1 [g/L in terms of TBTCI) died within 10 days. In the group exposed to 0.1 [g/L (0.1 [g/L in terms of TBTCI), about a half of them died within 15 days. Growth inhibition was observed for the surviving mussels.
- Effects on blue mussels exposed to tributyltin chloride were studied by Valkirs et al.⁴⁷⁾ As a result, 66-day LC₅₀ was calculated to be 0.97 [g/L.
- Effects on copepods exposed to tributyltin oxide were studied by U'ren³¹⁾. As a result, 144-day LC₅₀ was calculated to be 0.55 [g/L (0.60 [g/L in terms of TBTCI).

(3) Reproductive toxicity

(a) Fish

The outline of the reports on reproductive toxicity to fish obtained through JICST are mentioned below. Reliability assessment is not made by MoE.

- Effects on matured male and female red medakas given feed in which tributyltin oxide was mixed in the ratio of 1 [g/fish weight g/day were studied by Nirmala et al.⁴⁸⁾ As a result, a decrease in spawning frequency, survival rate of eggs, swim-up rate of larvae, and survival rate of juvenile fish was observed.

(b) Aquatic invertebrates

The reports on reproductive toxicity to aquatic invertebrates covered in the WHO Environmental Health Criteria²⁾ are summarized below.

- Effects on female copepods exposed to tributyltin oxide at 0.01, 0.05 or 0.1 $\mu\text{g/L}$ for 120 hours were studied by Johansen and Mohlenberg⁴⁹⁾. As a result, a significant fall in spawning was observed in the group exposed to tributyltin oxide at 0.01 $\mu\text{g/L}$ (0.01 $\mu\text{g/L}$ in terms of TBTCI) or over.
- Effects on *Gyraulax* exposed to tributyltin oxide at 0.001, 0.01, 0.1, 1, or 10 $\mu\text{g/L}$ were studied by Ritchie et al.⁵⁰⁾ As a result, the group exposed to 10 $\mu\text{g/L}$ (11 $\mu\text{g/L}$ in terms of TBTCI) were totally inhibited from spawning and died within five days. In the group exposed to 0.1 $\mu\text{g/L}$ (0.1 $\mu\text{g/L}$ in terms of TBTCI) or over, a fall in spawning was observed. No effect on spawning was observed in the group exposed to 0.01 $\mu\text{g/L}$ (0.01 $\mu\text{g/L}$ in terms of TBTCI).
- Effects on female copepods exposed to tributyltin chloride at 0.0125-0.5 $\mu\text{g/L}$ for 13 days were studied by Hall et al.⁵¹⁾ As a result, a significant fall in the survival rate of newborn larvae was observed in the group exposed to 0.1 $\mu\text{g/L}$, and a significant decrease in the number of eggs of a brood and deaths of newborn larvae were observed in the group exposed to 0.5 $\mu\text{g/L}$. No effect on the number of eggs of a brood was observed in the group exposed to 0.0125-0.2 $\mu\text{g/L}$, and the survival of newborn larvae was not affected in the group exposed to tributyltin chloride at 0.05 $\mu\text{g/L}$ or lower.

6. Literature search and reliability assessment concerning suspected endocrine disrupting effects on fish etc.

(1) Effects on fish

(a) *In vitro* test

Within the literature information of 1972-2000 obtained through TOXLINE, etc., no report was found on the *in vitro* test results on estrogenic or androgenic effects.

(b) Animal test (*in vivo* test)

Within the literature information of 1972-2000 obtained through TOXLINE, etc., it was found that the following reports showed suspected endocrine disrupting effects on fish, and its reliability was acknowledged in the reliability assessment carried out by MoE.

- Effects on sheepshead minnow exposed to bis-tributyltin oxide at 0.41, 0.66, 1.3, 3.2 or 5.4 $\mu\text{g/L}$ (respectively 0.45, 0.72, 1.4, 3.5 or 5.9 $\mu\text{g/L}$ in terms of TBTCI) for 180 days were studied by Manning et al.⁵²⁾ As a result, a fall in survival rate was observed in the F_0 generation of the group exposed to 0.66 $\mu\text{g/L}$ (0.72 $\mu\text{g/L}$ in TBTCI terms) or over, and all the fries died in the group exposed to 5.4 $\mu\text{g/L}$ (5.9 $\mu\text{g/L}$ in TBTCI terms) and in the F_1 generation of the group exposed to 3.2 $\mu\text{g/L}$ (3.5 $\mu\text{g/L}$ in TBTCI terms). No effect on reproduction (number of eggs produced, survival and growth of F_1 generation) was observed either in the F_0 generation of the group exposed to 3.2 $\mu\text{g/L}$ (3.5 $\mu\text{g/L}$ in TBTCI terms) or lower, or in the F_1 generation of the group exposed to 1.3 $\mu\text{g/L}$ (1.4 $\mu\text{g/L}$ in TBTCI terms) or lower.

Further, the following report on masculinization of flounders was presented in the 3rd symposium of the Japan Society of Endocrine Disrupter Research in December 2000, though only a summary of the lecture has been published. Its reliability has not been assessed.

- Effects on hereditary total female flounders given mixed feeds containing tributyltin oxide 0.1 or 1 $\mu\text{g/g}$ at the age of 35 to 100 days were studied by Shimazaki et al.⁵³⁾ As a result, sex reversal to male was observed at a rate of 25.7 % and 31.1 % respectively in the groups exposed to 0.1 and 1 $\mu\text{g/g}$.

(2) Effects on other aquatic organisms

Within the literature information of 1972-2000 obtained through TOXLINE, etc., the following reports showed suspected endocrine disrupting effects on aquatic organisms other than fish, and its reliability was acknowledged in the reliability assessment carried out by MoE.

- Effects on female rock shell (*Thais clavigera*) exposed to tributyltin chloride at 0.001, 0.003 or 0.021 $\mu\text{g/L}$ (measured values) for three months were studied by Horiguchi et al.⁵⁴⁾ As a result, imposex was observed in the groups exposed to 0.001 $\mu\text{g/L}$ or over.
- Effects on Ophiuroidea exposed to bis-tri-*n*-butyltin oxide at 0.01, 0.1 or 0.5 $\mu\text{g/L}$ for four weeks were studied by Walsh et al.⁵⁵⁾ As a result, inhibition of arm regeneration was observed in the groups exposed to 0.1 $\mu\text{g/L}$ (0.1 $\mu\text{g/L}$ in TBTCI terms) or over.
- Effects on shrimps exposed to tri-*n*-butyltin oxide at 0.1, 0.3, 0.5 or 5 $\mu\text{g/L}$ for 21 days were studied by Khan et al.⁵⁶⁾ As a result, delay in telson regeneration and molting was observed in the groups exposed to 0.1 $\mu\text{g/L}$ (0.1 $\mu\text{g/L}$ in TBTCI terms) or over.
- Effects on female dog-whelk exposed to tri-*n*-butyltin chloride at 0.5 $\mu\text{g/L}$ (calculated from the measured value of tin concentration) for 14 days were studied by Bryan et al.⁵⁷⁾ As a result, imposex was observed.
- Effects on fidler crabs exposed to bis-tri-*n*-butyltin oxide at 0.5 or 5.0 $\mu\text{g/L}$ for four weeks were studied by Weis and Kim⁵⁸⁾. As a result, deformities in regenerated claws and limbs were increased in the groups exposed to 0.5 $\mu\text{g/L}$ (0.5 $\mu\text{g/L}$ in TBTCI terms) or over.
- Effects on embryos of *Styrela plicata* (Tunicata) exposed to tributyltin chloride at 33, 326 or 3,255 $\mu\text{g/L}$ were studied by Cima et al.⁵⁹⁾ As a result, inhibition of development was observed in the groups exposed to 326 $\mu\text{g/L}$ or over.

7. Results, etc. of Screening endocrine disrupting effects in fish

(1) *In vitro* assays

(a) Competitive binding assay to medaka estrogen receptor

The binding affinities of TBTCI to medaka (*Oryzias latipes*) and human estrogen receptors were measured by competitive binding assay using the ligand binding domain of these estrogen receptors (α) fused with glutathione S-transferase (GST), expressed in *E. coli*, and radiolabelled estradiol as a ligand. At TBTCI concentration of more than 10^{-6} M, the release of the ligand from estrogen receptor depending on the concentration of TBTCI was observed with both receptors. There is not any difference in release curves between medaka and human estrogen receptors. These results, however, do not indicate that TBTCI replace the ligand specifically bound to estrogen receptor because there is the possibility that the ligand could be released by denaturing of estrogen receptor with TBTCI due to its strong protein denaturing ability. Then the denaturing of estrogen receptor was measured as a function of TBTCI by monitoring the enzyme activity of GST fused to the receptor, which is expected to be denatured with TBTCI in the same manner as the receptors. The similar relationship between the enzymatic activity and TBTCI concentration as the release of ligand was observed though the decrease of the enzyme activity begin at slight lower concentration than the release of ligand. These results strongly suggest that the release of ligand from estrogen receptors is caused by denaturing of the receptors by TBTCI.

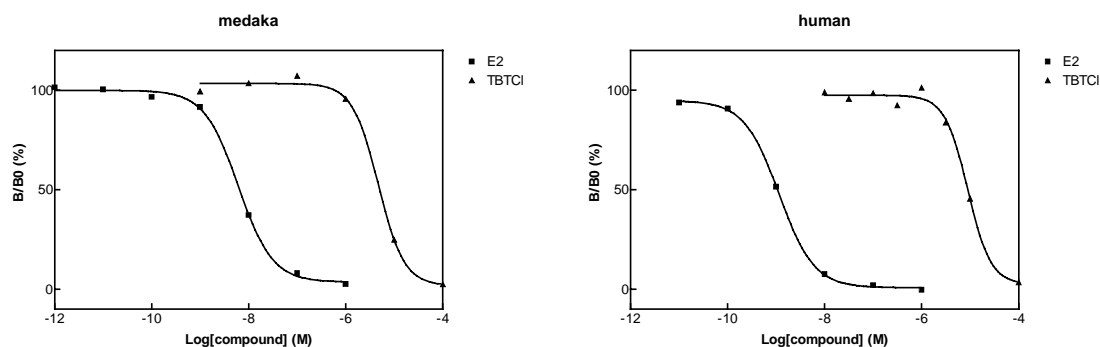


Fig. 4 Elimination of [³H]-estradiol from estrogen receptor by estradiol and TBTCI (left: medaka; right: human)

Table 3. Relative binding strength of TBTCI obtained from [3H]-estradiol elimination curves

Species	Relative binding strength (%)	
	Estradiol	TBTCI
Medaka	100	0.13
Human	100	0.013

(b) Reporter gene assay

The expression and reporter plasmids were transiently co-transfected to Hela cell exposed with TBTCI, and after incubation overnight the luciferase activity induced by transcriptional activation by TBTCI bound estrogen receptor was measured as a function of TBTCI concentration. No enhancement of the luciferase activity was observed over 10⁻¹² to 10⁻³ M of TBTCI, but the activity decreased from the basal value at more than 10 nM. The decreasing curve of luciferase activity from basal activity is similar to decreasing one of GST activity, which is the indication of denaturing of estrogen receptor. These results supported the finding that TBTCI denature proteins in cell including estrogen receptor and consequently inhibit of cell activity at these concentrations.

In conclusion, no data indicating that TBTCI bind specifically to estrogen receptor is obtained from competitive binding and reporter gene assays.

(2) *In vivo* studies using medaka

(a) Medaka vitellogenin assay

This study was conducted to assess the effects of tributyltin chloride (TBTCI) on vitellogenin (precursor of egg yoke protein) synthesis in medaka. About 3-month-old medaka (respectively 10 females and males/treatment) were exposed to TBTCI at the concentrations of 117, 269, 606, 1,640 and 4,000 ng/L (mean measured concentrations) under flow-through conditions for 21 days. 17 β -estradiol (E2, 100 ng/L) was tested as positive control. Daily observation was made to examine mortality and abnormal behavior and appearance during the exposure period. At the end of exposure, the livers of fish were removed, and vitellogenin concentration in each liver was measured.

Neither death nor particular symptom was observed during the exposure period. At the end of exposure, the hepatosomatic index (HSI) of male fish exposed to ≥ 269 ng/L was significantly higher than that in the controls. As for vitellogenin concentration, however, no statistically significant change was observed in both males and females in any treatment, as compared with that in the

controls (Fig.5).

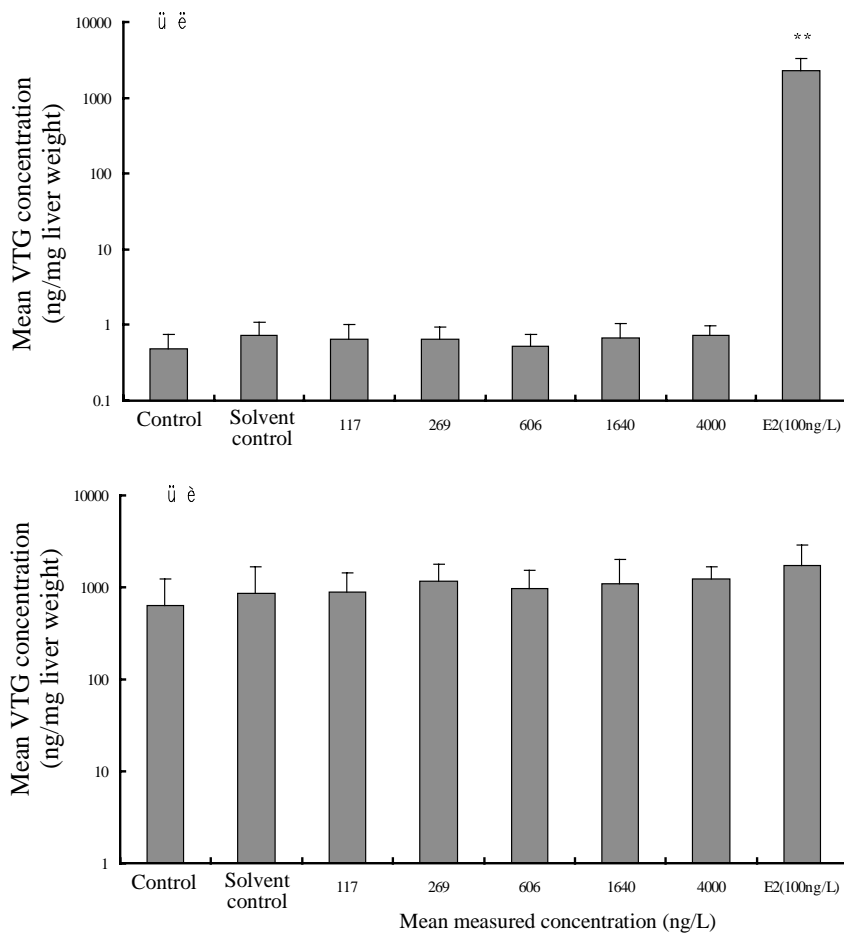


Fig. 5 Vitellogenin (VTG) concentrations in the livers of male and female medaka (*Oryzias latipes*) in the vitellogenin assay.

Data is shown as mean \pm standard deviation. ** denotes a significant difference at $p < 0.01$.

(b) Medaka partial life test

This test was conducted to evaluate endocrine disrupting effects of tributyltin chloride (TBTCI) on sexual differentiation of medaka. Medaka (60 eggs/treatment) were exposed to TBTCI at the concentrations of 20.1, 64.1, 205, 594 and 1,650 ng/L (mean measured concentrations) under flow-through conditions from fertilized eggs to 61-day posthatch. No significant effects were observed on hatching of embryos at the concentrations tested. However, posthatch mortality in the highest treatment (1,650 ng/L) increased markedly, and the cumulative mortality at 61-day posthatch was significantly higher than the control mortality. The growth of fish at 61-day posthatch were suppressed with increasing TBTCI concentrations, resulting in significant differences in the total length at 594 and 1,650 ng/L, and in the body weight at 1,650 ng/L. These results suggested that TBTCI would have lethal toxicity or growth-inhibitory effects on medaka larvae and juveniles at ≥ 594 ng/L. Based on an examination of the secondary sex characteristics of the surviving fish at 61-day posthatch, there were no significant differences in the sex ratio in any treatments, although more females than males were identified in the 594 ng/L and 1,650 ng/L treatments. Gonadal histology showed that histological abnormalities, such as hermaphroditism, were not observed in any treatments (Table 4). The HSI of fish at 61-day posthatch was increased

in 594 ng/L and 1,650 ng/L treatments, suggesting that TBTCI exerts hepatotoxicity at these concentrations. The hepatic vitellogenin concentrations in males at the end of exposure were significantly increased in all treatments relative to the solvent controls, but not to the controls. And there was no clear concentration-response relationship between the vitellogenin concentrations and the TBTCI treatments. It was not possible, therefore, to conclude that there was a clear vitellogenin induction in male medaka (Fig.6). The vitellogenin concentration in females did not show significant differences in any treatments.

As mentioned above, it was suggested that TBTCI would have chronically lethal toxicity or growth inhibitory effects on medaka at ≥ 594 ng/L TBTCI. In this test, however, it was not observed that TBTCI affected sexual differentiation of medaka by its endocrine disrupting effects.

Table 4. Sex ratios as determined by gross examination of secondary sex characteristics of medaka (*Oryzias latipes*) at 61- day posthatch and by their gonadal histology.

TBTCI concentration* (ng/L)	Secondary sex characteristics			Gonadal histology		
	N	Sex ratio (B:≡)		N	Number of fish	
					Testis	Ovary
Control	50	23 : 27		20	12	8
Solvent control	53	28 : 25		20	14	6
20.1	53	26 : 27		20	11	9
64.1	50	23 : 27		20	7	13
205	51	25 : 26		20	10	10
594	53	22 : 31		20	7	13
1,650	33	13 : 20		20	8	12

* Shown in mean measured concentrations.

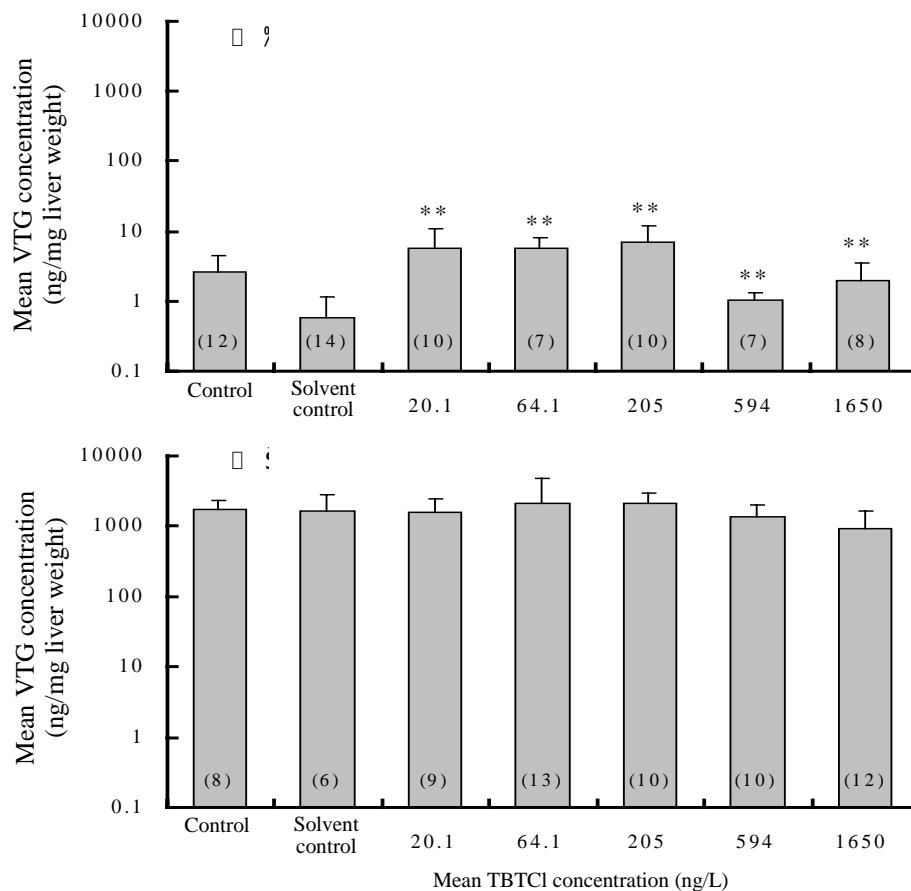


Fig. 6 Vitellogenin (VTG) concentrations in the livers of medaka (*Oryzias latipes*) at 61-day posthatch in each treatment. Data is shown as mean±standard deviation. Numbers in parentheses refer to number of fish. ** denotes a significant difference relative to the solvent controls at $p < 0.01$.

8. States of research, etc. for elucidating the effects of very low concentration TBT on snails such as rock shell

(1) Characteristics of imposex

Imposex of rock shell (*Thais clavigera*) was still observed in high frequency in the field tests carried out in 1996 to 1999 (See Appendix 2).

The word "imposex" is said to be derived from "superimposition of male characters on to females", and according to Smith⁶⁰ who used this word first, it usually refers to the formation of male genital organs, such as penis and deferent duct, in females of dioecious snails and to the female snails in which male genital organs have been formed. Based on the studies so far, the peculiar symptom to imposex is thought to be the masculinization of female snails⁶¹. In concrete, one or both of masculine genital organs (penis or deferent duct) are formed and developed, causing lower function of ovary (incomplete oogenesis) or transformation of ovary into testis, or transforming oviduct into prostate gland (swollen deferent duct, an organ found in male) in some cases^{60,62,63}. Serious imposex would cause a lowering or loss of spawning ability. Such a lowering or loss is known to occur in three patterns: i) blockade of vulva (opening for spawning) due to increased peripheral tissues following the formation of deferent duct⁶⁴; ii) lowering or loss of oogenesis ability, including transformation of ovary into tes-

tis⁶³); and (iii) cleavage of oviduct (incomplete closing in the process of growth) or impediment to copulation and spawning due to transformation of oviduct into prostate gland⁶⁵).

Imposex is an irreversible symptom, and if this has happened, recovery is impossible at the level of individuals^{62,66}. Symptoms such as formed penis or vulva blockade will never be resolved. At the population level, however, it is thought that, following the reduction of organic tin pollution, imposex symptom in a population will be relieved and the situation will be "recovered" with an increase in population⁶⁷. However, it cannot simply be discussed whether spawning inhibition by imposex will eventually be the main cause of a decrease in population of the species, because it is related with the ecology in their initial life history after hatch⁶⁸).

Also, it would be necessary to pay attention to the extremely high concentration of triphenyltin (TPT) in ovary or in oviduct (ovisac gland) containing degenerated ovisac lump⁵⁴. That is because the accumulation of organic tin compounds in eggs at a high concentration is suspected to have any bad effect on generation or hatch of eggs.

The reverse phenomenon to imposex, that is feminization of male snails, has never been observed in the fields⁶⁹).

(2) Substances causing imposex

Imposex is known to be induced by some kinds of organic tin compounds, such as TBT and TPT, almost peculiarly, even in a low concentration of 1 ng/L or so in the case of TBT, and independently from growth stage (age), when exposed to such organic tin compounds^{54,57,62,70,71}. Looking closely at chemical species of organic tin causing imposex, there are differences among species in terms of the extent of activity and the existence of effect^{57,70-72}. For example, TBT and tripropyltin (TPrT) are positive both in rock shell (*Thais clavigera*) and European dog-whelk (*Nucella lapillus*), but TPT effects are exactly opposite between both species^{56,69,71}. Recently, it was confirmed that imposex was caused by TPT in fresh water snails in Germany (J. Oehlmann, private correspondence). At present, these results are supposedly due to differences among species, and the causes for the differences have to be elucidated^{69,70}.

In what part of gastropods and in what mechanism would TBT and TPT cause imposex? What are their critical periods or threshold values? These questions are yet to be answered. So far, some hypotheses have been presented on the mechanism of inducing imposex⁹⁶⁻⁹⁸, as described below:

(3) Hypotheses about imposex induction mechanism

As to the mechanism of inducing imposex, there are three types of hypotheses: (i) aromatase inhibition theory⁷³); (ii) cerebral ganglion inhibition theory⁷⁴), and (iii) androgen excretion inhibition theory⁷⁵). Of them, the first (i) and the third (iii) theories are paying attention to steroid hormone, and the second (ii) to neurohormone, respectively. According to the first theory, TBT will inhibit aromatase, causing an increase in concentration of internal androgen (testosterone) significantly out of proportion to estrogen (estradiol), and the excessive androgen (testosterone) will be combined to its receptor, triggering the masculinization of female⁷³). In the third theory, TBT will, by inhibiting sulfate conjugation ability, restrain androgen (testosterone) and its metabolic product from being excreted as sulfate conjugator outside the body, causing an increase in concentration of internal androgen (testosterone) to trigger the masculinization of female⁷⁵). As for the second theory, naturally in female, penis formation inhibiting factor is excreted from cerebral ganglion against the penis formation accelerating factor from foot ganglion, but TBT will inhibit the excretion of penis formation inhibiting factor, causing the formation and growth of penis in female⁷⁴).

All these theories have been drawn by the help of a plural number of experiments, but they are not sufficient yet. As for the first theory, it is still doubtful, because of a reverse phenomenon in time series that, in experiment, penis formation preceded an increase in androgen (testosterone) concentration⁷³), in addition to the fact that the existence, as well as chemical structure, of androgen, estrogen and aromatase in gastropods has not been confirmed fully by their separation, refining, etc.

Recently, of the steroids detected from rock shell and ivory shell, five kinds were identified in high-resolution gas chromatography/mass spectrometry, but the analysis of their detailed functions is the task to be addressed in the future⁷⁶⁾. Also there is a report that, in the snail samples collected in the organotin-polluted sea area and the control sea area, their imposex symptoms did not correspond to aromatase effects, suggesting that a consistent trend about aromatase inhibition hypothesis is not always observed⁷⁷⁾.

Further, there are many doubtful points to be elucidated with regard to aromatase inhibition hypothesis, including structures and functions as well as information transmission system of steroid hormone receptors, possibility of TBT to inhibit aromatase syntheses or cause a loss of aromatase activity, interactions between TBT and steroid hormone receptors⁷⁸⁾, and whether similar phenomena are caused by other organic tin (for example, TPT) than TBT.

As for the third (iii) hypothesis, in view of the fact that their test was made in a short period using a high concentration of TBT, it cannot be denied that the imposex might be caused by acute toxicity, leaving vagueness in interpretation of test results.

As for the second (ii) hypothesis, it has been clarified that neuropeptide, together with its base array, excreted from visceral ganglion, cephalic ganglion or prostate gland of sea hares (*Aplysia kurodai*) and pond snails (*Limnaeidae*), is egg-laying hormone, ovulation hormone, or spawning hormone^{79,80)}. But it is not made clear what is the secretion factor from ganglion said to be involved in penis formation, thus further verification is difficult. While basic knowledge on neurohormone is still insufficient, any involvement of neuropeptide in causing imposex has attracted renewed attention when it was recently reported that APGW amide, a kind of neuropeptide excreted from cephalic ganglion of pond snail, promoted imposex of mud snail (*Ilyanassa obsoleta*)⁸¹⁾.

As mentioned above, there remain many tasks to be coped with in the study to elucidate imposex induction mechanism, and it is necessary to promote basic and applied studies on reproductive physiology of snails. In these studies, efforts must be made to find a solution to the simultaneous equations satisfying all the premises that imposex is caused almost peculiarly by some kinds of organic tin compounds, that imposex is caused by TBT at a very low concentration of 1 ng/L or so in the environmental water, and that imposex is a phenomenon observed peculiarly in snails.

Further, it is also necessary to study whether the mechanism inducing intersex thought to be a similar phenomenon to imposex, in *Littorina littorea*⁸²⁾ and masculinization of female in abalones⁸³⁾ may be regarded as the common mechanism to that of imposex, or what common and different points are between the two mechanisms.

9. Assessment results on endocrine disrupting effects of tributyltin (TBT)

(1) Effects on fish

Of the literature information of 1972-2000 obtained through TOXLINE, etc., no report was obtained on the *in vitro* test on estrogenic or androgenic effects of fish. In animal tests, effects on sheepshead minnow were examined, but in any exposure group, no effect on reproduction was observed⁵²⁾. On the other hand, though reliability assessment was not conducted, a report was obtained that TBT had an effect on sex differentiation of flounders, causing sex reversal of hereditary total females⁵³⁾.

Of MoE's *in vitro* test results, the medaka receptor binding assay showed that the binding was not high because the relative binding strength to estrogen receptor was about 1/1000, as compared with E₂. It was also strongly indicated that the binding could possibly be affected by TBTCl's denaturing activity.

Of MoE's screening results, in the male medaka vitellogenin assay, significant change was not observed at any concentration.

Also, in the medaka partial life cycle test, an increase in female rate was observed at TBTCl concentration of 594 ng/L or higher, but without any significant difference. Any physiological abnormality, such as hermaphrodite, was not observed, either. As for male medaka vitellogenin, a statistically

significant difference was observed at all CBTCI concentration sectors against auxiliary agent sector, but not against control sector. And the change in vitellogenin concentration did not show any clear dependence on TBTCI concentration. So, it was not concluded that any clear vitellogenin induction occurred in male bodies.

As mentioned above, from the results of MoE's various tests using medaka and from the literature information on which reliability assessment was carried out, any clear result was not obtained that tributyltin (TBT) compounds had endocrine disrupting effect on fish. However, there was a report that sex reversal was caused in flounders⁵³⁾, though it is not known whether the reversal was due to endocrine disruption or not. Further, it is not known yet whether such a sex reversal was a response peculiar to flounders liable to reverse sex in response to water temperature change or stress, whether it was due to difference in sensitivity among fish species, or whether it was due to difference in exposure methods (water exposure, feed exposure) or resultant exposure concentration, etc. The report also assumed that the masculinization was caused by the TBT activity as aromatase inhibitor, but the world's effort to elucidate the effect on aromatase was just started. So it is necessary to accumulate further scientific data on various fish species, including verification of reproducibility of sex reversal as observed in flounders.

(2) Effects on other aquatic organisms

As shown in Section 6(2), of the literature information of 1972-2000 obtained through TOXLINE, etc., in-water TBT concentrations suspected of having endocrine disrupting effects on aquatic organisms other than fish in the reported test results, of which reliability was confirmed, are 0.001 [g/L where imposex of female rock shell was observed⁵⁴⁾, 0.1 [g/L where inhibition of arm regeneration for *Ophioderma brevispina* was observed⁵⁵⁾, 0.1 [g/L where delay in telson regeneration and molting of shrimps was observed⁵⁶⁾, 0.5 [g/L where imposex of male dog-whelk was observed⁵⁷⁾, 0.5 [g/L where increased deformities in regenerated claws and limbs of fiddler crab were observed⁵⁸⁾, 326 [g/L where development inhibition of embryos of white sea squirt (*Styrela plicata*) was observed⁵⁹⁾, etc.

As mentioned above, there were many reports on suspected endocrine disrupting effects of TBT on aquatic organisms. The in-water concentration 0.001 [g/L where imposex was observed⁵⁴⁾, which was the Lowest-Observed-Effect-Concentration (LOEC), was equivalent to the detection limit value. The maximum No-Observed-Effect-Concentration (NOEC) and the Predicted-No-Effect-Concentration (PNEC) were below the detection limit value, therefore it was difficult to set NOEC and PNEC using the present analysis technology.

Tributyltin (TBT) compounds are thought to have effect on various aquatic organisms at very low concentration, but they have, in the first place, strong general toxicity, including denaturing activity at relatively low concentration, as shown in *in vitro* tests. In addition, scientific studies to elucidate what kinds of hormones and receptors exist and how they function in these invertebrates have just started, so that it is difficult to judge, at this point of time, whether or not the effects on reproduction and development of invertebrates as reported in the literature were caused by endocrine disrupting effects.

(3) Effects on mammals

On human health effects, MoE is now conducting various tests. In the *in vitro* tests using human cells, TBT's relative binding strength to estrogen receptor is not high at about 1/10,000, compared with that of E₂, and the possibility is strongly suggested that this binding could be attributed to denaturing activity. In the animal tests using rodents, as obtained in our literature search, it should be noted that no effect at very low concentration has been reported (See Appendix 3).

10. Assessment results and future challenges

Tributyltin (TBT) compounds are thought to have effects on various aquatic organisms at very low concentration, but it is appropriate to conclude that, from the present literature search and various test results using medaka, there was no clear results that TBT compounds have endocrine disrupting effect on fish, noting that it is not known whether or not the masculinization of flounders, as reported but not published yet, was due to endocrine disrupting effects.

MoE's present tests used medaka, an OECD subject fish species. No results on suspected masculinization of sheepshead minnows were observed, either. As a result, the possibility is very low that masculinization will be observed even if any full-life-cycle test is carried out as a confirmation test. At this point, therefore, it is thought that tributyltin (TBT) compounds have no endocrine disrupting effects on fish or their effects are extremely low. On the reproducibility of reports on masculinization of flounders, etc., however, it should be noted that further examination, including reassessment might be if necessary, waiting for the future accumulation of scientific knowledge and views.

Some reports were found on suspected endocrine disrupting effects on other aquatic organisms. But it was deemed difficult to judge, at this point of time, whether those effects were due to endocrine disrupting effects or not. As for the test methods for assessing endocrine disrupting effects on these invertebrates, no examination has been started yet at the OECD level. It is important, therefore, to promote research and studies to elucidate the endocrine system of snails, including rock shell, and to develop and establish the test methods for the assessment of endocrine disrupting effects on invertebrates, paying attention to the progress at the OECD etc.

11. Additional remarks

Because of their strong general toxicity and low degradability, tributyltin (TBT) compounds have attracted much attention internationally since late 1980s. In Japan, the manufacture, import and use of them are strictly controlled under the "Law Concerning the Examination and Regulation of Manufacture etc. of Chemical Substances", and related industries have practiced self-control on the use of TBT compounds under the guidance of the Ministry of Transport and the Fishery Agency. TBT compounds are not used any longer in antifouling agents for fishing nets and antifouling paints for ships. Instead of TBT paints, hydrolytic paints are now used for ships.

According to recent environmental monitoring surveys, however, TBT compounds are still detected, though in low concentration, in the water and aquatic organisms, and no significant improvement is seen in the bottom sediment. Among the causes and sources pointed out are TBT's low degradability and TBT-containing paints for ocean-going vessels⁵⁾.

In the light of the fact that, as mentioned before, effects such as imposex of rock shells are observed even at detection limit value, and imposex is still observed at high frequency in many ports and bays, further reduction of TBT compounds is needed. As for tributyltin (TBT) compounds contained in ship bottom paints, discussions are under way in the International Maritime Organization (IMO) to ban their use in ship bottom paints, aiming at the conclusion of an international treaty in 2001. Apart from promoting such internationally-coordinated compulsive regulations, it will be necessary to promote research and development of safe substitutes, too.

(Appendix 1)

1. International regulations, etc.

(1) IMO

Starting from mid 1980s, adverse effects of organic tin (especially tributyltin: TBT) compounds used in antifouling paints for ships on the marine environment have been taken up internationally as a big problem. In the 30th Marine Environment Protection Committee meeting (MEPC30) of the International Maritime Organization (IMO) in November 1990 was adopted the MEPC Resolution No.46 recommending the ban on the use of TBT-based antifouling ship paints for small-sized ships below 25 m in length, etc. After that, monitoring results mainly by developed countries and a report on performance, etc. of substitute paints by Japan were submitted, but they did not lead to any positive action to ban TBT-based antifouling ship paints.

In the MEPC38 held in July 1996, however, a proposal to ask for the necessity of world-wide regulations on the use of TBT was made by Japan, the Netherlands and Nordic countries, and the MEPC decided to include it in the working plan, setting up the Correspondence Group (CG) to gather opinions from countries prior to the MEPC40 which would start substantial discussions. Then, the examination works were carried out by the "Correspondence Group on the Reduction of Harmful Effects on the Use of Anti-fouling paints for Ships", centering on the Netherlands.

In the MEPC41 (March 1998), the final report by CG was submitted, and substantial deliberations were started based on the report. As a result, it was decided to set up a working group (WG) to start full-scale deliberations on the details. It was also approved to send the draft resolutions agreed on after deliberations to the 21st IMO General Assembly.

As a result, at the 21st IMO General Assembly (November 1999), the resolutions including the following contents were adopted:

- └ The MEPC should develop a legally binding framework (convention) to ban any new coating of organic tin-based antifouling ship paints on ships from January 1, 2003 on, and to ban those paints being coated on ships from January 1, 2008 on;
- └ Countries should encourage their national industries to develop, test and use alternative antifouling ship paints not imposing adverse effect on the marine environment;
- └ Countries should develop the methods of assessing antifouling paints, review their social and environmental effects, and promote scientific and technological studies on the effects of antifouling paints on the environment.

Further, at the general meeting, it was also agreed to hold a diplomatic meeting in 2001 in order to adopt the above-mentioned convention.

The discussions in the MEPC forums toward making up a convention on TBT were also continued, and at the MEPC43 (June 1999), the discussion was made on the concrete contents of the draft convention based on the proposal from the U.S.A. Then, through discussions at the MEPC44, the MEPC45 (October 2000) came to an agreement on the principal part of the treaty draft.

At the MEPC46 (April 2001), a broad agreement was reached on the draft treaty, though some details remained unsolved, and the convention is expected to be adopted at the diplomatic meeting scheduled in October, 2001.

(2) Europe and North America⁵⁾

With the MEPC Resolution No.46 as a turning point, European and American developed countries banned the use of TBT on small ships and coasting vessels. But they have not introduced regulations on the use of TBT-based paints on ocean-going vessels subject to IMO treaties, in the light of no alternative paint equivalent to TBT-based antifouling ship paints and a significant effect on the economy of shipowners.

Nordic countries are highly aware of the environmental pollution, and moving ahead toward the introduction of related water standards and zone restrictions. In the U.S.A., the Environment Protection Agency (EPA) is reported to have started the registration of substances to be used in the ship paints alternative to TBT, but no report is received that the use of TBT is totally banned or ceased.

2. Regulations outside Japan

Regulations on use of organic tin-based antifouling ship paints outside Japan

Regulations	USA	Canada	Australia	New Zealand	France	United Kingdom	Netherlands, Ireland	Other EU countries	Sweden	Many other non-EU countries	South Africa
Ships less than 25 m in length: Ban on all organic tin-based paints (except aluminum)	☒	☒		☒	☒						
Ships less than 25 m in length: Ban on all organic tin-based paints (without exception)			☒								
Ships less than 25 m in length: Ban on TBT-based paints (without exception)							☒	☒	☒	☒	☒
Ban on the use of paints containing tri-form organic tin compounds for ships less than 25m in length and fish culture equipment & materials						☒					
Ships of 25 m or over in length: TBT-based paints may be used only in containers of 20l							☒	☒			☒
Ships of 25 m or over in length: Elution speed of 4 [g TBT/cm ² /day or lower	☒	☒							☒		
Ships of 25 m or over in length: Elution speed of 5 [g TBT/cm ² /day or lower			☒								
Registration of all antifouling paints	☒	☒	☒	☒		☒	☒		☒		☒
TBT-based paints may be used only by authorized engineers	☒										
All antifouling paints are registered as biocide agents. Approval by Advisory Committee is necessary for their sales and use.						☒					
Paints containing tri-form organic tin compounds may be sold only in containers of 20l or more. Total tin content in copolymer must be 7.5 % or less, or 2.5 % or less as free tin.						☒					

* Organic tin-based paints are totally banned in some sea areas.

Cited from the Shipbuilding Research Association of Japan (1998) The FY1997 report on research studies for the prevention of marine pollution (relating to anti-fouling paints for ships) at the 76th standard study group meeting.

This table was prepared in 1997, and re-examination is now under way.

(Appendix 3) Summary of effects of tributyltin (TBT) on mammals

1. General toxicity

(1) Acute toxicity

Mentioned below are the findings on acute toxicity to mammals in the literature information of 1966-2000 obtained through MEDLINE, etc. and in the World Health Organization (WHO) Environmental Health Criteria. Reliability assessment has not been made by MoE.

- Effect on the rats exposed to a single oral administration of tributyltin acetate respectively at 160, 208, 270.4, 351.5 and 456.98 mg/kg was studied by Attahiru et al.⁸⁴⁾ As a result, the median lethal dose LD₅₀ after 24 hours was calculated as 297.54 mg/kg.
- In WHO's Environmental Health Criteria, oral administration acute toxicity tests are reported by Truhaut et al.⁸⁵⁾, Funahashi et al.⁸⁶⁾, Schweinfurth⁸⁷⁾, Sheldon⁸⁸⁾, Klimmer⁸⁹⁾, Polster and Halacka⁹⁰⁾, and Pelikan and Cerny⁹¹⁾, using tributyltin chloride, tributyltin oxide, tributyltin fluoride, tributyltin acetate, tributyltin benzoate, oleic tributyltin, tributyltin linoleate, abietic tributyltin, tributyltin naphthenate, and tributyltin laurate as test articles, and rats and/or mice as test animals²⁾. As a result, the median lethal dose LD₅₀ was in the range of 46-234 mg/kg.

(2) Chronic toxicity

Mentioned below are the findings on chronic toxicity to mammals in the literature information of 1966-2000 obtained through MEDLINE, etc. Reliability assessment has not been made by MoE.

- Effects on the golden hamsters exposed to a single oral administration of tributyltin chloride respectively at 29.6, 44.4, 66.7, 100.0, 150.0 and 225.0 mg/kg were studied by Takagi et al.⁹²⁾ As a result, the median lethal dose LD₅₀ after two weeks was calculated at 146.9 mg/kg in male, and 172.0 mg/kg in female.
- Effects on the Wistar rats administered mixed feeds containing tributyltin oxide respectively at 0.5, 5 and 50 ppm for two years were studied by Wester et al.⁹³⁾ As a result, inhibited increase in body weight, decrease in body weight, and increase in mortality were observed in the 50 ppm administered group. No effect was observed on testis or ovary.

(3) Reproductive toxicity

Mentioned below are the findings on reproductive toxicity to mammals in the literature information of 1966-2000 obtained through MEDLINE, etc. Reliability assessment has not been made by MoE.

- Effects on Wistar rats orally administered tributyltin chloride at 5, 9, 15 or 25 mg/kg/day in the 7-15th day of pregnancy were studied by Itami et al.⁹⁴⁾ As a result, significant decrease in weight of female embryos was observed in the group administered 5 mg/kg/day or more. In the group administered 25 mg/kg/day, 7 out of 10 mother rats died, and all embryos were found dead.
- Effects on SD rats orally administered tributyltin chloride at 1 or 5 mg/kg/day in the 6-20th day of pregnancy were studied by Gardlund et al.⁹⁵⁾ As a result, increased quantity of motion of young, motion-stimulation increase by *p*-Antafemin, and impediment to space learning ability were observed in the group administered 1 mg/kg/day or more. In the group administered 5 mg/kg/day, slight inhibition of body weight increase of young was observed.
- Effects on the Long-Evans rats orally administered tributyltin oxide at 2.5, 5.0, 10, 12 or 16 mg/kg/day in the 6-20th day of pregnancy were studied by Crofton et al.⁹⁶⁾ As a result, in the group administered 10 mg/kg/day or more, decrease in the number of young, low value in body weight of young, inhibited increase in body weight of young after weaning, delay in vagina opening time, decrease in brain weight, and effect on behavior (quantity of motion, and sound-surprise response) were observed. In the group administered 12 mg/kg/day or more, teratogenicity of young was observed.

- Effects on the Wistar rats orally administered tributyltin chloride at 8.1, 12.2 or 16.3 mg/kg/day in the 0-7th day of pregnancy were studied by Harazano et al.⁹⁷⁾ As a result, decrease in feed intake by mother rats, inhibition of body weight increase, increase in pregnancy failure rate, and low values of embryo body weight were observed in the group administered 12.2 mg/kg/day or more.
- Effects on the Wistar rats orally administered tributyltin chloride at 8.1, 16.3 or 32.5 mg/kg/day in the 0-3rd day of pregnancy, and at 8.1, 16.3, 32.5 or 65.1 mg/kg/day in the 4-7th day of pregnancy were studied by Harazono et al.⁹⁸⁾ As a result, in the case of administration in the 0-3rd day of pregnancy, decrease in pregnancy rate of mother rats and low values of embryo body weight were observed in the group administered 16.3 mg/kg/day or more, and embryo death rate after implantation increased significantly in the group administered 32.5 mg/kg/day. In the case of administration in the 4-7th day of pregnancy, damage rate of embryos after implantation increased in the group administered 16.3 mg/kg/day, and significant decrease in the number of surviving embryos and low values of male embryo body weight were observed in the group administered 32.5 mg/kg/day or more.
- Effects on Wistar rats orally administered tributyltin chloride at 25 or 50 mg/kg/day in the 7-9th day, 50 or 100 mg/kg/day in the 10-12th day, or 25, 50 or 100 mg/kg/day in the 13-15th day of pregnancy were studied by Ema et al.⁹⁹⁾ As a result, in the case of administration in the 7-9th day of pregnancy, inhibition of increase in body weight of mother rats was observed in the group administered 25 mg/kg/day or more, and marked decrease in the number of survival embryos was observed in the group administered 50 mg/kg/day. In the case of administration in the 10-12th day of pregnancy, inhibition of increase in body weight of mother rats, decrease in the number of embryo survival, and decrease in body weight of embryos were observed in the group administered 100 mg/kg/day. And, palate cleavage was observed in 11 of 82 embryos. In the case of administration in the 13-15th day of pregnancy, palate cleavage was observed in the group administered 25 mg/kg/day or more. In the group administered 100 mg/kg/day, inhibition of increase in body weight of mother rats and decrease in body weight of embryos were observed, but no effect on survival rate of embryos was observed.
- Effects on the Wistar rats orally administered tributyltin chloride at 40 or 80 mg/kg/day in the 7-8th day of pregnancy were studied by Ema et al.¹⁰⁰⁾ As a result, inhibition of increase in body weight of mother rats, and decrease in the number of embryo survival were observed in the group administered 40 mg/kg/day or more.

(4) Other toxicity

Of the literature information of 1966-2000 obtained through MEDLINE, etc., the findings on the behavioral effects of TBT on mammals are mentioned below. Reliability assessment has not been made by MoE.

- Effects on the male Wistar rats exposed to a single administration of 1.6 or 3.3 mg/kg tributyltin chloride into abdominal cavity were studied by Ema et al.¹⁰¹⁾ As a result, inhibition of increase in body weight and significant decrease in the quantity of motion in the nighttime were observed in the group administered 3.3 mg/kg.

2. Literature search and reliability assessment concerning suspected endocrine disrupting effects on mammals etc.

Of the literature information of 1966-2000 obtained through MEDLINE, etc., no report on estrogenic or androgenic effects of tributyltin (TBT) to mammals was obtained.

Mentioned below are the reports on *in vitro* tests of TBT effects on thymic cells of rats and animal tests of TBT effects on immunological or endocrine systems of rats. Reliability assessment has not been made by MoE.

(1) *In vitro* test

- Effects on thymic cells of rats exposed to tributyltin chloride were studied by Snoeij et al.¹⁰²⁾ As a result, energy metabolism was markedly affected in the exposure group of 0.1 μ mol/L or more, and disorder of cell membranes and collapse of cells were observed in the exposure group of 1 μ mol/L or more.

(2) Animal tests (*in vivo* tests)

- Effects on the male Fischer 344 rats orally administered tributyltin oxide at 1.25, 2.5, 5 or 10 mg/kg/day for 10 days were studied by Smialowicz et al.¹⁰³⁾ As a result, decrease in thymus weight was observed in the group administered 2.5 mg/kg/day or more.
- Effects on the Long-Evans rats in the 5th day of birth administered tributyltin oxide into abdominal cavity at 2.0, 3.0, 4.0 or 5.0 mg/kg were studied by O'Callaghan and Miller¹⁰⁴⁾. As a result, decrease in brain weight was observed in the group administered 2.0 mg/kg/day or more, and decrease in thymus weight was observed in the group administered 4.0 mg/kg/day or more.
- Effects on the Wistar rats orally administered tributyltin acetate at 1, 2, 4, 8 or 16 mg/kg/day in the 7-10th day of pregnancy were studied by Noda et al.¹⁰⁵⁾ As a result, thymus weight decreased depending on dose in the group administered 4 mg/kg/day or more. In the group administered 16 mg/kg/day, increase in body weight of mother rats was inhibited, and all the embryos were found dead in 5 of 10 rats. Further, the number and weight of survival embryos decreased markedly, and palate cleavage increased.
- Effects on Wistar rats administered mixed feeds containing tributyltin oxide at 5, 20, 80 or 320 ppm for 4 or 6 weeks were studied by Krajnc et al.¹⁰⁶⁾ As a result, in the case of 4-week administration, shrink of mesentery lymph node and decrease in male thymus weight were observed in the group administered 20 ppm or more; decrease in weight of mesentery lymph node, inhibition of increase in male body weight, decrease of lymphocytes, and decrease in female thymus weight in the group administered 80 ppm or more; and inhibition of increase in female body weight and decrease of lymphocytes in the group administered 320 ppm. In the case of 6-week administration, concentration of insulin in serum decreased in the administration groups of 20 and 80 ppm; and decrease in concentration of TSH and T4 in serum, increase in concentration of LH in serum, decrease in the number of pituitary TSH producing cells, increase in the number of LH producing cells, decrease in reactivity of TSH to TRH, and rise in reactivity of LH to LHR were observed in the group administered 80 ppm.

References

- 1) Japan Environment Agency (1998) Strategic Programs on Environmental Endocrine Disrupters'98 - SPEED'98.
- 2) World Health Organization(1990)Tributyltin compounds, Environmental Health Criteria 116.
- 3) Uemura, S., Kawamura, H., Tsuji, M., Tomita, S. and Maeda, S. (1998) Cyclopedia of agricultural chemical toxicity, Sanseido.
- 4) U.S. National Library of Medicine(1999)Hazardous Substances Data Bank
- 5) The Shipbuilding Research Association of Japan (1998) The FY1997 report on research studies for the prevention of marine pollution (related to antifouling paints for ships at the 76th standard study group meeting, 182pp.
- 6) Safety assessment study committee on bis-tributyltin oxide (TBTO) in foods (Environmental Health Bureau, Japanese Ministry of Health and Welfare) (1985) Report on safety assessment study report on bis-tributyltin oxide (TBTO) in foods, 7-8, April 24, 1985.
- 7) Stab,J.A.,Traas,T.P.,Stroomberg,G.,van Kesteren,J.,Leonards,P.,van Hattum,B.,Brinkman,U.A.T., and Cofino,W.P.(1996)Determination of organotin compounds in the foodweb of shallow freshwater lake in the Netherlands. Arch. Environ. Contam. Toxicol.,31,319-328.
- 8) Slesinger,A.E. and Dresser,I.(1978)The environmental chemistry of three organotin chemical, in Report of the organotin workshop, New Orleans,Louisiana,17-19 February,1978,115-162.
- 9) Thain,J.E.,Waldock,M.J. and Waite,M.E.(1987)Toxicity and degradation studies of tributyltin(TBT) and dibutyltin(DBT) in the aquatic environment. in Proceedings of the Organotin Symposium, Oceans'87 Conference, Halifax, Nova Scotia, Canada,28 September-1 October, 1987,New York, The Institute of Electrical and Electronics Engineers,4,1398-1404.
- 10) Seligman,P.F.,Valkirs,A.O. and Lee,R.F.(1986)Degradation of tributyltin in marine and estuarine waters. in Proceedings of the organotin symposium,Oceans'86,Conference, Washington,D.C.,USA,23-25 September, 1986,New York, The Institute of Electrical and Electronics Engineers, Inc.,4,1189-1195.
- 11) Maguire,R.J.,Tkacz,R.J.,Chau,Y.K.,Bengert,G.A. and Wong,P.T.S.(1986)Occurrence of organotin in water and sediment in Canada. Chemosphere,15,253-274.
- 12) Maguire,R.J.,Wong,P.T.S. and Rhamey,J.S.(1984)Accumulation and metabolism of tri-*n*-butyltin cation by a green alga, *Ankistrodesmus falcatus*. Can.J.Fish,Aquat.Sci.,41,537-540.
- 13) Laughlin,R.B.,French,W. and Guard,H.E.(1986)Accumulation of bis(tributyltin)oxide by the marine mussel *Mytilus edulis*. Environ.Sci. Technol.,20,884-890.
- 14) Waldock,M.J.,Thain,J.E. and Miller,D.(1983)The accumulation and depuration of bis (tributyltin)oxide in oyster. A comparison between the Pacific oyster(*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*), Copenhagen, International Council for the Exploration of the Sea(ICES),6,Report No.C.M.1983/E.52).
- 15) Cheng,Z. and Jensen,A.(1989)Accumulation of organic and inorganic tin in blue mussel, *Mytilus edulis*, under natural conditions. Mar.Pollut.Bull.,20,281-286.
- 16) Allen,A.J.,Quitter,B.M. and Radick,C.M.(1980)The biocidal mechanism of controlled release bis (tributyltin) oxide in *Biomphalaria glabrata*. in ed. Baker,R., Controlled release of bioactive materials, New York, London, Academic Press,399pp.

- 17) Ward,G.S.,Cramm,G.C.,Parrish,P.R.,Trachman,H. and Slesinger,A.(1981)Bioaccumulation and chronic toxicity of bis(tributyltin)oxide(TBTO): Tests with a saltwater fish. in ed.. Branson, D.R. and Dickson,K.L., Proceedings of the Fourth Conference on Aquatic Toxicology and Hazard Assessment, Philadelphia, American Society for Testing and Materials,183-200. (ASTM STP No.737).
- 18) Bressa,G.,Cima,L.,Canova,F. and Caravello,G.U.(1984)Bioaccumulation of tin in the fish tissues (*Liza aurata*). in Proceedings of the International Conference on Environmental Contamination, London, July, Geneva, International Register of Potentially Toxic Chemicals, United Nations Environment Programme,812-815.
- 19) Short,J.W. and Thrower,F.P.(1986)Accumulation of butyltins in muscle tissue of chinook salmon reared in sea pens treated with tri-*n*-butyltin. in Proceedings of the Organotin Symposium Ocean'86 Conference, Washington,DC.,USA,23-25 September, 1986,New York, The Institute of Electrical and Electronics Engineers, Inc.,4,1177-1181.
- 20) Tsuda,T.,Nakanishi,H.,Aoki,S. and Takebayashi,J.(1987)Bioconcentration and metabolism of phenyl tin chlorides in carp. Water Res.,21,949-953.
- 21) Tsuda,T.,Nakanishi,H.,Aoki,S. and Takebayashi,J.(1986)Bioconcentration of butyltin compounds by round crucian carp.Toxicol.Environ.Chem.,12,137-143.
- 22) Short,J.W. and Thrower,F.P.(1987)Toxicity of tri-*n*-butyl-tin to chinook salmon, *Oncorhynchus tshawytscha*. adapted to seawater. Aquaculture,61,193-200.
- 23) Thain,J.E.(1983)The acute toxicity of bis(tributyl tin)oxide to the adults and larvae of some marine organisms. Copenhagen, International Council for the Exploration of the Sea(ICES). 5,Report No. C,M,1983/E.13.
- 24) Kakuno,A. and Kimura,S.(1987)Acute toxicity of bis(tributyltin)oxide to girella(*Girella punctata*). J. Bull. Tokai Reg.Fish.Res.Lab.,123,41-44.
- 25) Shimizu,A. and Kimura,S.(1987)Effect of bis(tributyltin)oxide on gonadal development of a salt-water goby, *Chasmichthys dolichognathus*. Exposed during maturing period. Bull. Tokai Reg.Fish.Res.Lab, 123,45-49.
- 26) RIVM(1989)Investigation into the toxicity of TBTO for a number of freshwater organisms. in ed. Mathijssen-Spiekman,E.A.M.,Canton,J.H. and Roghair,C. J., Bilthoven, National Institute of Public Health and Environmental Hygiene, 48,Report No.668118.001.
- 27) Linden,E.,Bengtsson,B.-E.,Svanberg,O. and Sundstrom,G.(1979)The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (*Alburnus alburnus*) and the harpacticoid *Nitcora spinipes*. Chemosphere,8,843-851.
- 28) Temmink,J.H.M. and Everts,J.W.(1987)Comparative toxicity of tributyltin-oxide(TBTO) for fish and snail. in Proceedings of the Seventh World Meeting of the ORTEP-Association. Amsterdam,7-8 May, 1987,Vissingen-Oost, The Netherlands, ORTEP-Association,6-20.
- 29) Plum,H.(1981)Comportement des composes organostanniques vis-à-vis de l'environnement. Inf, Chim., 220,135-139.
- 30) Goodman,L.R.,Cripe,G.M.,Moody,P.H. and Halsell,D.G.(1988)Acute toxicity of malathion, tetrabromo-bisphenol-A, and tributyltin chloride to mysids(*Mysidopsis bahia*) of three ages. Bull.Environ.Contam. Toxicol., 41,746-753.

- 31) U'Ren,S.C.(1983)Acute toxicity of bis(tributyltin)oxide to a marine copepod. Mar. Pollut. Bull.,14,303-306.
- 32) Meador,J.P.(1986)An analysis of photobehavior of *Daphnia magna* exposed to tributyltin. in Proceedings of the Organotin Symposium Ocean'86 Conference,Washington,DC.,USA,23-25 September, 1986, New York, The Institute of Electrical and Electronics Engineers,Inc.,4, 1213-1218.
- 33) Walsh,G.E.(1986)Organotin toxicity studies conducted with selected marine organisms at EPA's environmental research laboratory, Gulf Breeze, Florida. in Proceedings of the Organotin Symposium Ocean'86 Conference,Washington,DC.,USA,23-25 September, 1986,New York, The Institute of Electrical and Electronics Engineers, Inc.,4,1210-1212.
- 34) Davis,R.J.,Fletcher,R.L. and Furtado,S.E.J.(1984)The effects of tributyltin compounds on spore development in the green alga *Enteromorpha intestinalis* (L.) Link. in Proceedings of the 6th International Congress on Marine Corrosion and Fouling. Athens, September,1984,557-565.
- 35) Walsh,G.E.,McLaughlan,L.L.,Lores,E.M.,Louie,M.K. and Deans,C.H.(1985)Effects of organotins on growth and survival of two marine diatoms, *Skeletonema costatum* and *Thalassiosira pseudonana*, Chemosphere, 14,383-392.
- 36) Salazar,S.M.(1985)The effects f bis(tri-*n*-butyltin)oxide on three species of marine phytoplankton. San Diego, California, Naval Ocean Systems Center,21, Technical Report No. 1039.
- 37) Wong,P.T.S.,Chau,Y.K.,Kramar,O. and Bengert,G.A.(1982)Structure-toxicity relationship of tin compounds on algae.Can.J.Fish.Aquat.Sci.,39,483-488.
- 38) Beaumont,A.R. and Newman,P.B.(1986)Low levels of tributyltin reduce growth of marine micro-algae. Mar.Pollut.Bull.,17,457-461.
- 39) Floch,H.,Deschiens,R. and Floch,T.(1964)Sur les proprietes molluscicides de l'oxide et de l'acetate de tributyletain(prophylaxie des bilharzioses).Bull.Soc.Pathol.Exot,57,454-465.
- 40) Deschiens,R. and Floch,H.(1968)Les proprietes molluscicides du chlorure et de l'acetate de triphenyltain dans le carde de la prophylaxie des bioharzioses.C.R.Acad.Sci.Paris,255,1236-1237.
- 41) Bokranz,A. and Plum,H.(1975)Industrial manufacture and use of organotin compounds. Bergkamen, Federal Republic of Germany, Schering AG,33.
- 42) Seinen,W.,Helder,T.,Vernij,H.,Penninks,A. and Leeuwangh,P.(1981)Short term toxicity of tri- *n*-butyltinchloride in rainbow trout(*Salmo gairdneri* Richardson) yolk sac fry. Sci. Total Environ.,19,155-166.
- 43) Wester,P.W.,Canton,J.H.,Van Iersal,A.A.J.,Kranjc,E.I. and Vaessen,H.A.M.G.(1988)The toxicity of bis(tri-*n*-butyltin)oxide(TBTO) and di-*n*-butyltindichloride(DBTC) in the small fish species *Oryzias latipes*(medaka) and *Poecilia reticulata*(Guppy). Utrecht, The Netherlands, University of Utrecht (Wester, P.W.,Ph.D.Thesis).
- 44) Weis,J.S.,Weis,P. and Wang,F.(1987)Developmental effects of tributyltin on the fiddler crab, *Uca pugilator*, and the medaka, *Fundulus heteroclitus*. in Proceedings of the Organotin Symposium Ocean'87 Conference, Halifax, Nova Scotia,Canada,28 September-1 October, 1987,New York, The Institute of Electrical and Electronics Engineers,Inc.,4,1456-1460.
- 45) His,E. and Robert,R.(1985)Developpement des veligeres de *Crassostrea gigas* dans le Bassin d'Arcachon, etudes sur les mortalites larvairres.Rev.Trav.Inst.Peches Marit.,47,63-88.

- 46) Beaumont,A.R. and Budd,M.D.(1984)High mortality of the larvae of the common mussel at low concentrations of tributyltin.Mar.Pollut.Bull.,15,402-405.
- 47) Valkirs,A.O.,Davidson,B.M. and Seligman,P.F.(1987)Sublethal growth effects and mortality to marine bivalves from long-term exposure to tributyltin. Chemosphere,16,201-220.
- 48) Nirmala,K.,Oshima,Y.,Lee,R.Imada,N.,Honjo,T. and Kobayashi,K.(1999)Transgenerational toxicity of tributyltin and its combined effects with polychlorinated biphenyls on reproductive processes in Japanese medaka(*Oryzias latipes*).Environmental Toxicology and Chemistry,18,4,717-721.
- 49) Johansen,K. and Mohlenberg,F.(1987)Impairment of egg production in *Acartia tonsa* exposed to tributyltin oxide. Ophelia,27,137-141.
- 50) Ritchie,L.S.,Lopez,V.A. and Cora,J.M.(1974)Prolonged applications of an organotin against *Biomphalaria glabrata* and *Schistosoma mansoni*. in Molluscicides in schistosomiasis control. New York, London, Academic Press,77-88.
- 51) Hall,L.W.,Bushong,S.J.,Hall,W.S. and Johnson,W.E.(1988)Acute and chronic effects of tributyltin on a Chesapeake Bay copepod. Environ.Toxicol.Chem.,7,41-46.
- 52) Manning,C.S., Lytle,T.F.,Walker,W.W. and Lytle,J.S.(1999)Life-cycle toxicity of bis(tributyltin) oxide to the sheepshead minnow(*Cyprinodon variegatus*). Arch.Environ.Contam.Toxicol.,37, 258-266.
- 53) Shimazaki, Y., Kitano, T., Oshima, Y., Imada, N. and Honjo, T. (2000) Masculinization of flounders by tributyltin, Collected summaries of lectures at the 3rd symposium of Japan Society of Endocrine Disrupter Research, A-3-1, 65.
- 54) Horiguchi, T.,Shiraishi,H.,Shimizu,M.,Yamazaki,S. and Morita,M.(1995)Imposex in Japanese gastropods(Neogastropoda and Mesogastropoda):Effects of tributyltin and triphenyltin from antifouling paints. Marine Pollution Bulletin,31,4-12,402-405.
- 55) Walsh,G.E.,McLaughlin,L.L.,Louie,M.K.,Deans,C.H. and Lores,E.M.(1986)Inhibition of arm regeneration by *Ophioderma brevispina*(Echinodenmata, Ophiuroidea) by tributyltin oxide and triphenyltin oxide. Ecotoxicology and Environmental Safety,12,95-100.
- 56) Khan,A.,Weis,J.S.,Saharig,C.E. and Polo,E.(1993)Effect of tributyltin on mortality and telson regeneration of grass shrimp, *Palaemonetes pugio*. Bull.Environ.Contam.Toxicol.,50, 152-157.
- 57) Bryan,G.E.,Gibbs,P.E. and Burt,G.R.(1988)A comparison of the effectiveness of tri-*n*-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk, *Nucella lapillus*. J.Mar.Biol.Ass.U.K.,68,733-744.
- 58) Weis,J.S. and Kim.K.(1988)Tributyltin is a teratogen in producing deformities in limbs of the fiddler crab, *Uca pugnator*. Arch.Environ.Contam.Toxicol.,17,583-587.
- 59) Cima,F.,Ballarin,L.,Bressa,G.,Martinucci,G. and Burighel,P.(1996)Toxicity of organotin compounds on embryos of a marine invertebrate(*Styrela plicata*;Tunicata). Ecotoxicology and Environmental Safety, 35,174-182.
- 60) Smith,B.S.(1971)Sexuality in the American mud snail, *Nassarius obsoletus* Say. Proc. Malacol. Soc. Lond., 39, 377-378.
- 61) Matthiessen,P. and Gibbs,P.E.(1998)Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environmental Toxicology and Chemistry, 17, 1,37-43.
- 62) Gibbs,P.E.,Bryan,G.W.,Pascoe,P.L. and Burt,G.R.(1987)The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin(TBT) contamination. J. Mar. Biol. Ass. .K.,67, 507-523.

- 63) Gibbs,P.E.,Pascoe,P.L. and Burt,G.R.(1988)Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. J.Mar.Biol.Ass.U.K.,68,715-731.
- 64) Gibbs,P.E. and Bryan,G.W.(1986)Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. J. Mar. Biol. Ass. U.K., 66, 767-777.
- 65) Gibbs,P.E.,Bryan,G.W.,Pascoe,P.L. and Burt,G.R.(1990)Reproductive abnormalities in female *Ocenebra erinacea*(Gastropoda) resulting from tributyltin-induced imposex. Journal of the Marine Biological Association of the United Kingdom, 70,3, 639-656.
- 66) Bryan,G.W.,Gibbs,P.E.,Hummerstone,L.G and Burt,G.R.(1986)The decline of the gastropod *Nuceella lapillus* around south-west England: Evidence for the effect of tributyltin from antifouling paints.J.Mar.Biol.Ass.U.K.,66,611-640.
- 67) Gibbs, P.E. and Bryan, G.W.(1996)TBT-induced imposex in neogastropod snails: masculinization to mass extinction, in Tributyltin: case study of an environmental contaminant ed. by S.J. de Mora, Cambridge University Press, 212-236.
- 68) Horiguchi, T. (2000) II. Present state and causative substances of endocrine disrupting effects to wild life/shellfish, Endocrine disrupting substances in water environment (edited by Kawai, S. and Koyama, J.), Koseisha-Koseikaku, Tokyo, 54-72.
- 69) de Fur,P.L.,Crane,M.,Ingersoll,C. and Tattersfield,L.ed.(1999)Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment, SETAC Press, 303pp.
- 70) Horiguchi,T.,Shiraishi,H.,Shimizu,M. and Morita,M.(1997)Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. Environmental Pollution,95,1,85-91.
- 71) Horiguchi, T., Cho, H. H., Shiraishi, H., Shibata, Y., Morita, M. and Shimizu, M. (1997) Effects of 18 kinds of organic tin and steroid-hormone on imposex of rock shell, Collected summaries of lectures at Japanese Society of Fisheries Science autumn meeting (1997), 741, 105.
- 72) Hawkins,L.E. and Hutchinson,S.(1990)Physiological and morphogenetic effects of monophenyltin trichloride on *Ocenebra erinacea*. British Ecology Society Symposium, Southampton,England,UK,26-27 September, Funct. Ecol., 4,3, 449-454.
- 73) Bettin,C.,Oehlmann,J. and Stroben,E..(1996)TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. Helgolaender Meeresuntersuchungen,50,3,299-317.
- 74) Feral,C. and Le Gall,S.(1983)The influence of a pollutant factor (TBT) on the neurosecretory mechanism responsible for the occurrence of a penis in the females of *Ocenebra erinacea*. in 'Molluscan Neuro-endocrinology',ed. by J. Lever and H.H. Boer, North Holland Publishing, 173-175.
- 75) Ronis,M.J.J. and Mason,A.Z.(1996)The metabolism of testosterone by the Periwinkle (*Littorina littorea*) *in vitro* and *in vivo*: Effects of tributyl tin. Mar. Environ. Res., 42, 161-166.
- 76) Lu, H., Horiguchi, T., Shiraishi, H., Shibata, Y., Abo, M., Okubo, A. and Yamazaki, S. (2001) Identification and determination of steroid-hormone in seawater snails by chromatography/mass spectrometry, Analytical Chemistry, 50, 247-255.
- 77) Morcillo,Y. and Porte,C.(1999)Evidence of endocrine disruption in the imposex-affected gastropod *Bolinus brandaris*. Environ. Res. 81, 4,349-354.

- 78) Yamabe, Y., Hoshino, A., Imura, N., Suzuki, T. and Himeno, S. (2000) Enhancement of androgen-dependent transcription and cell proliferation by tributyltin and triphenyltin in human prostate cancer cells. *Toxicology and Applied Pharmacology*, 169, 177-184.
- 79) Chiu, A. Y., Hunkapiller, M. W., Heller, E., Stuart, D. K., Hood, L. E. and Strumwasser F. (1979) Purification and primary structure of the neuropeptide egg-laying hormone of *Aplysia californica*. *Proc. Natl. Acad. Sci. USA*, 76, 12, 6656-6660.
- 80) Ebberink, R. H. M., Loenhout, H., van Geraerts, W. P. M. and Joosse, J. (1985) Purification and amino acid sequence of the ovulation neurohormone of *Lymnaea stagnalis*. *Proc. Natl. Acad. Sci.*, 82, 7767-7771.
- 81) Oberdorster, E. and McClellan-Green, P. (2000) The neuropeptide APGWamide induces imposex in the mud snail, *Ilyanassa obsoleta*. *Peptides*, 21, 9, 1323-1330.
- 82) Bauer, B., Fioroni, P., Ide, I., Liebe, S., Oehlmann, J., Stroben, E., and Watermann, B. (1995) TBT effects on the female genital system of *Littorina littorea* : a possible indicator of tributyltin pollution. *Hydrobiologia*, 309, 15-27.
- 83) Horiguchi, T., Kojima, M., Kaya, M., Shiraishi, H., Morita, M., Takiguchi, N., Cho, H. H., Hirose, H. and Shimizu, M. (2000) Ovo-testis and disturbed reproductive cycle in the giant abalone, *Haliotis madaka*: Possible linkage with organotin contamination in a site of population decline. *Mar. Environ. Res.*, 50, 223-229.
- 84) Attahiru, U. S., Iyaniwura, T. T., Auda, A. O. and Bonire, J. J. (1991) Acute toxicity studies tri-*n*-butyltin and triphenyltin acetates in rats. *Vet. Hum. Toxicol.*, 33, 6, 554-556.
- 85) Truhaut, R., Chauvel, Y., Anger, J.-P., Phu Lich, N., Van Den Driessche, J., Guesnier, L. R. and Morin, N. (1976) Contribution a l'etude toxicologique et pharmacologique de l'oxide de tributyletain (OTBE). *Eur. J. Toxicol.*, 9, 31-40.
- 86) Funahashi, N., Iwasaki, I. and Ide, G. (1980) Effects of bis(tri-*n*-butyltin)oxide on endocrine and lymphoid organs of male rats. *Acta Pathol. Jpn.*, 30, 955-966.
- 87) Schweinfurth, H. (1985) Toxicology of tributyltin compounds. *Tin Uses*, 143, 9-12.
- 88) Sheldon, A. W. (1975) Effects of organotin anti-fouling coatings on man and his environment. *J. Paint Technol.*, 47, 54-58.
- 89) Klimmer, O. R. (1969) The use of organic tin compounds from the viewpoint of experimental toxicology. *Arzneimittelforschung*, 19, 934-939.
- 90) Polster, M. and Halacka, K. (1971) Contributions to the health-toxic problems of some anti-microbially used organo-tin compounds. *Ernahrungsforschung*, 16, 527-535.
- 91) Pelikan, Z. and Cerny, E. (1968) The toxic effects of tri-*n*-butyltin compounds on white mice. *Arch. Toxikol.*, 23, 283-292.
- 92) Takagi, S., Mano, H., Tsunoda, M., Nakadaira, H., Endoh, K. and Yamamoto, M. (1992) Acute toxicity of tri-*n*-butyltin chloride (TBTC) in the Syrian golden hamster. *Tohoku J. Exp. Med.*, 166, 3, 309-319.
- 93) Wester, P. W., Krajnc, E. I., Van Leeuwen, F. X. R., Loeber, J. G., Van der Heijden, C. A., Vaessen, H. A. M. G. and Helleman, P. W. (1990) Chronic toxicity and carcinogenicity of bis(tri-*n*-butyltin) oxide (TBTO) in the rat. *Fd. Chem. Toxic.*, 28, 3, 179-196.
- 94) Itami, T., Ema, M., Amano, H., Murai, T. and Kawasaki, H. (1990) Teratogenic evaluation of tributyltin chloride in rats following oral exposure. *Drug Chem. Toxicol.*, 13, 4, 283-295.

- 95) Gardlund,A.T.,Archer,T.,Danielsson,K.,Danielsson,B.,Fredriksson,A.,Lidqvist,N.G.,Lidstrom,H. and Luthman,J.(1991)Effects of prenatal exposure to tributyltin and trihexyltin on behaviour in rats. *Neurotoxicol.Teratol.*,13,1,99-105.
- 96) Crofton,K.M.,Dean,K.F.,Boncek,V.M.,Rosen,M.B.,Sheets,L.P.,Chernoff,N. and Reiter,L.W.(1989) Prenatal or postnatal exposure to bis(tri-*n*-butyltin)oxide in the rat. Postnatal evaluation of teratology and behavior. *Toxicology and Applied Pharmacology*,97,113-123.
- 97) Harazono,A.,Ema,M. and Ogawa,Y.(1996)Pre-implantation embryonic loss induced by tributyltin chloride in rats. *Toxicol.Lett.*,89,3,185-190.
- 98) Harazono,A.,Ema,M. and Ogawa,Y.(1998)Evaluation of early embryonic loss induced by tributyltin chloride in rats. Phase- and dose-dependent antifertility effects. *Arch. Environ. Contam.Toxicol.*, 34,1,94-99.
- 99) Ema,M.,Kurosaka,R.,Amano,H. and Ogawa,Y.(1995)Further evaluation of the developmental toxicity of tributyltin chloride in rats. *Toxicology*,96,3,195-201.
- 100) Ema,M.,Kurosawa,R.,Amano,H. and Ogawa,Y.(1995)Comparative developmental toxicity of tributyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. *J.Appl.Toxicol.*,15, 4,297-302.
- 101) Ema,M.,Itami,T. and Kawasaki,H.(1991)Changes of spontaneous motor activity of rats after acute exposure to tributyltin chloride. *Drug Chem.Toxicol.*,65,8,651-655.
- 102) Snoeij,N.J.,Punt,P.M.,Penninks,A.H. and Seinen,W.(1986)Effects of tri-*n*-butyltin chloride on energy metabolism, macromolecular synthesis, precursor uptake and cyclic AMP production in isolated rat thymocytes. *Biochim.Biophys.Acta*,852,234-243.
- 103) Smialowicz,R.J.,Riddle,M.M.,Rogers,R.R.,Luebke,R.W. and Copeland,C.B.(1989) Immunotoxicity of tributyltin oxide exposed as adults or pre-weanlings. *Toxicology*,57,1, 97-111.
- 104) O'Callaghan,J.P. and Miller,D.B.(1988)Acute exposure of the neonatal rat to tributyltin results in decreases in biochemical indicators of synaptogenesis and myelinogenesis. *J. Pharmacol. Exp.Ther.*, 246,1,394-402.
- 105) Noda,T.,Morita,S.,Yamano,T.,Shimizu,M.,Nakamura,T.,Saitoh,M. and Yamada,A.(1991) Teratogenicity study of tri-*n*-butyltin acetate in rats by oral administration. *Toxicol.Lett.*,55, 1.109-115.
- 106) Krajnc,E.I.,Wester,P.W.,Loeber,J.G.,van Leeuwen,F.X.R.,Vos,J.G.,Vaessen,H.A.M.G. and van der Heijden,C.A.(1984)Toxicity of bis(tri-*n*-butyltin)oxide in the rats.1.Short-term effects on general parameters and on the endocrine and lymphoid system. *Toxicology and Applied Pharmacology*,75,363-386.