

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

1. Background

In its SPPED (Strategic Programs on Environmental Endocrine Disrupters) '98¹⁾ published in May, 1998, Japanese Environment Agency, now Ministry of the Environment (MoE), listed 67 substances suspected to have endocrine disrupting effects. Since then, national environmental field surveys have been carried out by MoE. In October 1999, the meeting of Advisory Committee on Environmental Endocrine Disrupters (chairman: Dr. Tsuguyoshi Suzuki, professor emeritus of the University of Tokyo) was held, and it was recommended that risk assessments should be initiated, while hearing expert opinions, starting with the four substances (i.e. tributyltin, nonylphenol, 4-octylphenol and di-*n*-butyl phthalate) classified as priority substances for risk assessment, etc., based on the environmental field survey and literature search results.

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

This draft report describes the results of the risk assessment of nonylphenol on fish, etc., together with the test results for detecting endocrine disrupting effects on fish carried out by MoE as well as outcome of its literature search and reliability assessment, environmental surveys, etc.

As for nonylphenol's effects on human health, MoE is now conducting tests using rodents. An exposure survey (e.g. diet study) is now being planned. Another risk assessment on the part of human health will subsequently be made based on their results.

Nonylphenol has two types of the isomers, straight and branched. What is mainly detected in the environment and has relatively strong endocrine disrupting effects is found to be branched 4(or *p*)-nonylphenol, judging from chemical properties and literature search, etc. Therefore, the tests used 4-nonylphenol standard products (isomer mixtures, branched) from Kanto Kagaku, refined by removing decylphenol, etc. from the mixtures (branched) for industrial use. The literature search and the environmental surveys, too, were carried out, targeting branched isomers.

Episodes concerning how endocrine disrupting effects came to be suspected

As a start to draw attention to endocrine disrupting effects of nonylphenol, two episodes are wellknown:

- () Abnormal proliferation of breast cancer cells due to nonylphenol released from test apparatus.

In 1991, in a test by Soto, A. M. et al²⁾ of U.S.A. to proliferate breast cancer cells, hyperplasia was observed in the samples not administered estrogen, too. The cause was identified as nonylphenol released from test apparatus, which has weak estrogenic effects.

- () Appearance of bisexual fish individuals and existence of nonylphenol in river water.

In the River Lea in southern England, bisexual individuals of fish were observed. To investigate the causes, Sumpter and Jobling³⁾ measured vitellogenin concentrations in rainbow trout and nonylphenol concentrations in river water, mainly in the downstream of sewage treatment plant in the plural number of rivers. As a result, it was suggested that one of the causes could be alkylphenols, especially nonylphenol from detergents used for cleansing wool at textile mills.

2. Properties, uses, production, etc.

(1) Chemical structure, properties, etc.

(a) Chemical structure

Alkylphenol is synthesized through the Friedel-Crafts' reaction that combines phenols (C_6H_5OH) and olefins (C_nH_{2n} : unsaturated chain hydrocarbon with a double bond). When alkyl chain (C_nH_{2n+1}) is long, para (*p*- or 4-) isomers (thermal stability is high) are predominant although ortho (*o*- or 2-) isomer and *o*, *p*-di-alkyl isomers are produced, too. Meta (*m*- or 3-) isomers have the highest thermal stability, so that a little amount of meta-isomers are also produced in heat treatment.

As raw material of nonylphenol, mixtures of 5 kinds of propylene ($CH_3CH=CH_2$) trimers are used, and as raw material of octylphenol, mixtures of 2 kinds of isobutene ($(CH_3)_2C=CH_2$) dimers. It is easy to synthesize alkyl chains from (olefin or straight chain derivatives, but its demand is small⁴⁾.

There are 170 kinds of nonylphenol isomers theoretically, and 22 kinds of isomer mixtures by GC-MS techniques^{5,6)}. Each nonylphenol isomer is given a number (CAS RN or CAS No.) by Chemical Abstract Service (CAS), but seemingly there are confusions in citation of those numbers^{7,8)}. According to the CAS Secretariat⁹⁾, CAS No.25154-52-3 denotes nonylphenol including various isomers, CAS No.104-40-5 straight nonylphenol (*p*-nonylphenol), and CAS No.84852-15-3 branched nonylphenol (*p*-nonylphenol, branched).

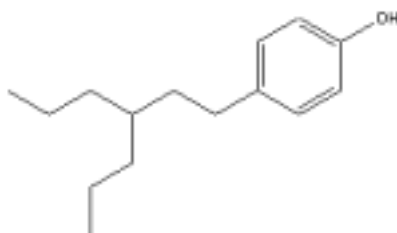


Fig. 1 An example of structural formulas of 4(or *p*)-nonylphenol, branched¹⁰⁾

(b) Major properties of nonylphenol⁷⁾

Molecular formula	: $C_{15}H_{24}O$
Molecular weight	: 220.34g/mole
Appearance	: Transparent or light straw-colored, high-viscosity liquid; slight phenolic odor
Specific gravity	: 0.95 (20 °C)
Melting point	: approx. -8 °C
Vapor pressure	: 0.3Pa or lower (25 °C)
Aquatic solubility	: 6mg/L (20 °C)
Log Kow	: 4.48

(2) Uses, production, etc.

(a) Alkylphenols

Four kinds of alkylphenols are produced industrially in Japan: nonylphenol, octylphenol, butylphenol, and dodecylphenol. Among them, nonylphenol and octylphenol are produced in large quantity in particular¹¹⁾.

Alkylphenols are used as raw materials for alkylphenol ethoxylates, a nonionic surface active agent¹¹⁾. Alkylphenol ethoxylates are degraded in the environment, resulting in the formation of alkylphenols.

For surface active agents, nonylphenol and octylphenol are used in the ratio of about 4:1¹²⁾.

(b) Nonylphenol

Nonylphenol is a kind of alkylphenol and has many isomers. In Japan, two companies are producing nonylphenol, and their total production was 16,800 tons¹³⁾, 17,400 tons¹⁴⁾ and 16,500 tons (surveyed by the Ministry of Economy Trade and Industry) respectively in 1998, 1999 and 2000.

Nonylphenol is used, as raw materials, for surface active agents (anionic surface active agent, nonionic surface active agent), ethyl cellulose stabilizers, oil soluble phenyl resins, esters, etc. It is also used, as processed articles, for detergents, oil varnishes, rubber auxiliaries and vulcanization accelerators, antioxidants and corrosion inhibitors for petroleum products, sludge generation inhibitors for petroleum, etc.¹⁵⁾

According to a report by the European Commission⁷⁾ in 1997, the production of nonylphenol in the European Union was 73,500 tons, of which 3,500 tons were exported. Import of nonylphenol into the EU was 8,500 tons in 1997. Of the total consumption 78,500 tons in the EU in 1997, 47,000 tons (about 60 %) were used as raw materials for nonylphenol ethoxylates, 29,000 tons (about 37 %) for plastics, resins and stabilizers and 2,500 tons (about 3 %) for aromatic oxims.

(c) Alkylphenol ethoxylates

Production of alkylphenol ethoxylates in Japan started in 1952. At present, about 30 companies are engaged in the production^{11,15)}. The production in 1998 was about 46,850 tons, including mixtures (about 34,600 tons if calculated in terms of 100 % alkylphenol ethoxylate according to the survey by Japan Surfactant Industry Association). Of this production, about 85 % was accounted for by nonylphenol ethoxylates, and the remainder by octylphenol ethoxylates, etc.¹¹⁾

Further, the production of alkylphenol used as raw material for alkylphenol ethoxylates was about 8,240 tons in 2000, equivalent to a half of the production of nonylphenol¹⁶⁾. The other half was used as raw material for other uses than surfactants, such as alkylphenol phosphite, etc. The consumption of alkylphenol ethoxylates in Japan was about 23,900 tons in 1998, the breakdown of which is shown below in Table 1¹¹⁾.

Table 1 Consumption of alkylphenol ethoxylates in Japan (1998)

Synthetic rubber & plastic industry	4200tons	17.6%
Textile industry	4000tons	16.7%
Metal processing	3300tons	13.8%
Institutional cleansing	2300tons	9.6%
Laundry cleaning	1400tons	5.9%
Dyestuff, pigments, paints & ink	1100tons	4.6%
Food processing industry	900tons	3.8%
Agricultural industry	800tons	3.4%
Paper & pulp industry	700tons	2.9%
Petroleum oil & fuel industry	600tons	2.5%
Construction & civil engineering	600tons	2.5%
Drugs & cosmetics	500tons	2.1%
Leather processing industry	100tons	0.4%
Others	3400tons	14.2%

3. Releases to the environment

(1) Releases of nonylphenol ethoxylates

- No natural occurrences of nonylphenol ethoxylates (NPEs) are known. Their presence in the nature is, therefore, solely a consequence of human activities.
- In Japan, they are supposedly released mainly by textile, metal processing, institutional cleansing, and laundry cleaning, etc. (see Section 2. Properties, uses, production, etc.), but no data on the amount of the release is available.
- According to the survey results by Environment Canada/Health Canada ¹⁷⁾, the release of nonylphenol (NP) and nonylphenol ethoxylates (NPEs) in the industrial production and use was 96.5 tons in 1996. Major sources of release were the release into rivers by surfactant producers (25-60 tons/year) and the release into the air, land surface or underground by industrial users of detergents, etc.(25-60 tons/year). Other releasers were paint producers (5-9.999 tons/year), producers of industrial and domestic detergents (0.1-4.999 tons/year), paper & pulp industry (0.1-4.999 tons/year), etc. (Figures for the release are shown in total of NPs and NPEs). It is conceivable that releases from households are significant, too, although the survey did not cover them.
- According to the survey results by the European Commission ⁷⁾, total release of nonylphenol ethoxylates on a continental basis is estimated at about 108 tons/day. Apart from the release from unknown sources which accounted for about 25 % of the total release, a little less than 50 % of the total was released by industrial users of detergents, etc., followed by textile industry (about 15 %) and leather processing industry (about 7 %). The release into water area by these four sources, including the unknown sources, is estimated to account for about 95 % of the total release. It is also estimated that most of the release goes into water area through effluent treatment facilities. Concerning the release from households into water area, it is included in the figures of the release from production process under the EU self-regulation on domestic use of detergents (the release of nonylphenol ethoxylates from production process is estimated at about 1 % of the total).

(2) Releases of nonylphenol

- No direct occurrences of nonylphenol (NP) in the nature are known. Its presence in the nature is, therefore, solely a consequence of human activities.
- In Japan, nonylphenol is generated through degradation of nonylphenol ethoxylates released mainly from textile, metal working, institutional cleansing, laundry cleaning, etc. (see Section 2. Properties, uses, production, etc.), but no data on the amount of the release is available.

- According to the survey results by the European Commission ⁷⁾, it is estimated that total release of nonylphenol in continental EU is about 3.0 tons/day, and about 95 % of the total is generated from nonylphenol ethoxylates released into waste water treatment facilities. The remainder (about 5 % of the total) is supposed to be released into the environment in the process of producing nonylphenol ethoxylates. Most of the release is estimated to go into water area.

4. Behavior in the environment

(1) Model calculation on distributions in the environment

- In the studies conducted by MoE ¹⁸⁾, taking domestic and foreign literature information into consideration, water and land areas were set from the mesh data covering the whole country (land area: $3.63 \cdot 10^{11} \text{ m}^2$, water area: $1.23 \cdot 10^{11} \text{ m}^2$), and relative concentrations (predicted distribution factors) of nonylphenol in respective media (e.g. water, sediment, suspended matters, and aquatic organisms) were obtained, using fugacity model (level I) assuming that distributions among respective media correspond to the property values of nonylphenol (e.g., distribution factor indicating the ratio of concentrations in sediment and those in water, bioconcentration factor (BCF) indicating the ratio of concentrations in water and those in aquatic organisms, etc.). As a result, it was estimated that there was a tendency that the concentrations of nonylphenol in sediments and suspended matters were 10^2 - 10^4 times higher than those in other media (e.g., water, organisms),

(2) Literature information, etc.

The literature information obtained through JICST and the reports by Environment Canada/Health Canada on behaviors in the environment are stated below. No reliability assessment is made by MoE.

(a) Distribution in the environment

- According to model calculations by Environment Canada/Health Canada ¹⁷⁾, 58-73 % of the nonylphenol released into water area is distributed in water, 27-41 % in sediment, and only a little (1 % or lower) in air and soil.
- According to the survey on several kinds of fish in 8 rivers flowing into Lake Biwa conducted by Tsuda, et al ^{19,20)}, bioaccumulation factor (BAF) of nonylphenol in omnivorous (mainly herbivorous) fishes, such as common minnow and sweet fish (Japanese ayu), was 21-31, and BAF in fish-eating black bass was 21, showing no tendency that fish species in a higher trophic level would show a higher value in BAF.
- According to the surveys conducted in the Glatt river in Switzerland, by Ahel, et al ²¹⁾, nonylphenol concentrations in the bodies of 3 kinds of fish (omnivorous, animal-eating, and fish-eating) were ND (<0.03)-1.6 mg/kg (dry weight), and those of wild ducks (herbivorous) living in the same water area was ND<0.03)-1.2 mg/kg (dry weight).
- As for the dilution immediately after the release, Environment Canada/Health Canada calculated predicted environmental concentration (EEV: Estimated Exposure Value) by dividing release concentrations by 10 as a dilution factor, for release sources of nonylphenol and nonylphenol ethoxylates ¹⁷⁾. The dilution factor of 10 is not a certain figure, but they assume it is applicable in the close neighborhood of release sources. The European Commission, too, uses a dilution factor of 10 in calculating predicted environmental concentration (PEC) in the downstream of release sources, taking dilution factor and distribution factor of suspended matters into consideration ⁷⁾.

(b) Degradability in the environment, etc.

- It is reported that the biodegradation mechanism of alkylphenol ethoxylates involves an initial shortening of ethoxy groups through aerobic sludge treatment in effluent treatment systems, etc., leading to the production of intermediates, such as nonylphenol diethoxylate, nonylphenol

monoethoxylate, nonylphenol dicarboxylate and nonylphenol monocarboxylate, and then to the production of nonylphenol through anaerobic sludge treatment. For example, nonylphenol ethoxylate will be degraded into nonylphenol through pathways as shown in Fig.2 ⁸⁾.

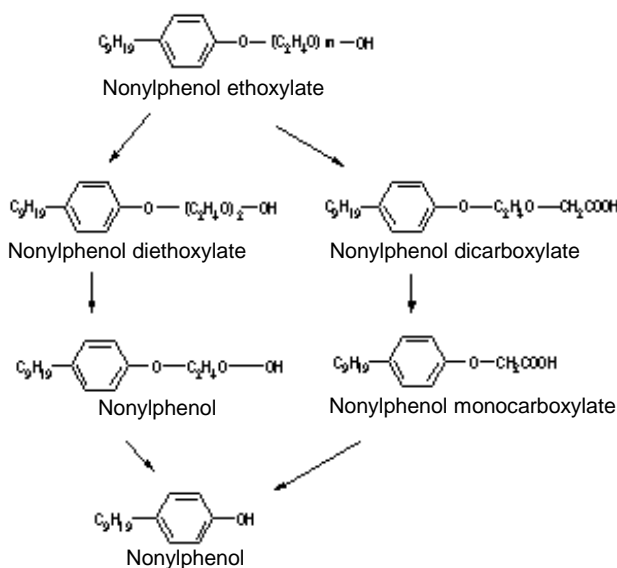


Fig.2 Degradation process of nonylphenol ethoxylate ⁸⁾

(a) Degradability of nonylphenol ethoxylates

- As for the biogradability of nonylphenol ethoxylate (chain length: 9), it is reported that, in degradability tests (OECD301D (closed bottle test) and 301E (revised OECD screening test)) and aerobic effluent treatment simulation test (coupled units test), initial degradation was observed, but full degradation did not occur, resulting in the production of hardly degradable substances ⁷⁾.
- In the biodegradation tests by Ahel ²²⁾, half-life obtained from the concentration decreases of nonylphenol ethoxylate (chain length: 1) in effluent-treated water was 47 days at 4°C, and 11 days at 20 . In a similar test in river water, half-life was about 3 days at 20 . In these tests, production of mainly nonylphenol carboxylates was observed.

(b) Degradability of nonylphenol

- Judging from the results of degradability tests (OECD301B (CO₂ production test) and OECD301F (Manometric Respirometry test)) in accordance with OECD Guideline, nonylphenol is reported to be not ready biodegradable, but inherently biodegradable ⁷⁾.
- In the case where degradation of nonylphenol was measured in terms of the quantity of CO₂ produced, hardly any degradation was observed even after 32 days ⁷⁾.
- In the degradation test of nonylphenol at 16°C using lake water in Canada conducted by Sundaram and Szeto ²³⁾, half-life was 16.3-16.5 days in lidded vessels, and 2.5 days in lidless vessels.
- Likewise, in the degradation test of nonylphenol using river water in Switzerland conducted by Ahel ²²⁾, half-life at 20 °C was 12 days.
- Degradation speed of nonylphenol in the air was calculated at 0.3 days ⁷⁾.

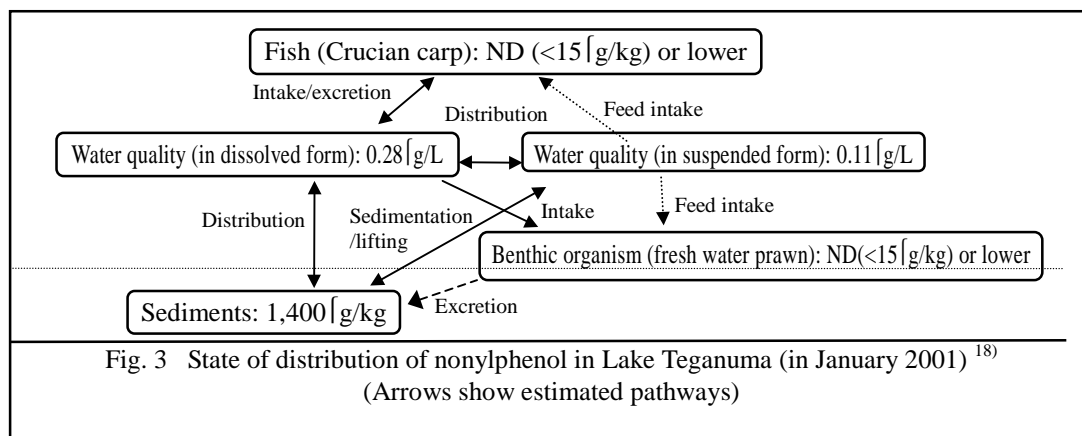
(3) Test and survey results on behavior in the environment

According to the survey by MoE¹⁸⁾ on the concentration of nonylphenol ethoxylates and nonylphenol in river water flowing into Lake Teganuma, and in the lake water and bottom sediments in the lake, the proportion of molar concentration of nonylphenol ethoxylates (chain length: 1-17) to that of nonylphenol was 4-7 : 1 in flowing water, and 2-4 : 1 in lake water, showing a trend that the lower the proportion of nonylphenol ethoxylates, the shorter the chains of nonylphenol ethoxylates (chain length: 1-13). In lake sediments, the proportion was 2/3 : 1, showing that the molar concentration of nonylphenol was higher than that of nonylphenol ethoxylates, and the chains of nonylphenol ethoxylates became even shorter (chain length: 1, 2).

From the results mentioned above, it was supposed that nonylphenol ethoxylates released into water area gradually degraded in the water, increased their hydrophobic property as their chains become shorter, and tended to be more easily absorbed in suspended matters or in organic substances in the sediments.

The state of distribution of nonylphenol in Lake Teganuma is as shown in Fig.3.

In the water, nonylphenol exists in dissolved form or in suspended form absorbed in suspended matters. As the concentration in suspended form depends on the mass of suspended matters, the concentration in dissolved form was higher than that in suspended form in this survey.



5. Fate in the body

(1) Literature information, etc.

The literature information obtained through JICST and the reports by the European Commission or Environment Canada/Health Canada on the dynamics in the body are stated below. Reliability assessment for the following has not been made by MoE.

- Studies were made by Lewis and Lech²⁴⁾ on half-life of nonylphenol (kind of isomer unknown) in the body of rainbow trout. As a result, half-life in the body was 19-20 hours when exposed to¹⁴⁾ C-marked nonylphenol at concentration of 18 [g/L for 8 hours. Though metabolic pathways are not known in detail, it is supposed that the nonylphenol was excreted into urea by sulfate conjugation as is generally the case with phenols.
- Bioconcentration factor (BCF) (concentration in body/concentration in water) of chemicals is usually obtained by growing aquatic organisms in the water at certain concentrations in the laboratory. Reported BCF values for nonylphenol include 88-116 in rainbow trout²⁵⁾, 90-330 in carp²⁶⁾, 167 in red medaka²⁰⁾, 191-253 in blue gill²⁷⁾, 271-984 in fathead minnow^{27,28)}.
- BCF values obtained by Tsuda, et al^{19,20)} from outdoor survey in Lake Biwa was a figure lower than the values obtained in the laboratory: 2 or lower in crucian carp, 15 in blue gill, 15-22 in carp, 21 in sweet fish, 21 in black bass, 25 in dark chub, and 31 in pale chub.

(2) Test and survey results on fate in the body

According to the survey report by MoE¹⁸⁾, with regard to 4-nonylphenol (branched) taken in from feeds and accumulated in the body, when given the mixed feeds containing 89 [g/kg 4-nonylphenol (branched) under the conditions of 2.8 [g/L 4-nonylphenol (branched) concentration in the water, BAF in carp was 124 (concentration in body/concentration in water), but no significant difference ($p < 0.05$) was observed against the BCF (113) in the test section without 4-nonylphenol (branched) in the feeds under the same conditions of concentration in the water. Further, these values were similar to the existing reported BCF values (90-330) for carp.

On the other hand, the following calculation formula was used in order to estimate the intake of nonylphenol from water and feeds:

$$dC_b/dt = k_1 C_w - (F C_f + k_2) C_b$$

* N.B.) C_b : Concentration in the body ([g/kgw), C_w : Concentration of dissolved chemical in the water ([g/L), k_1 : intake rate constant (L/kgw/day), k_2 : excretion rate (1/day), F : absorption rate of chemical from feeds (%/100), C_f : concentration of chemical in feeds ([g/kg), F : feed intake per day (kg/kgw/day)

(note: This formula expresses concentration changes in the body. The term of intake from feeds ($F C_f$) was added to the OECD formula, $dC_b/dt = k_1 C_w - k_2 C_b$)

The calculation results indicated that the nonylphenol intake from feeds was several percent or less of the intake from water. Consequently, it was supposed that most of nonylphenol in the bodies of aquatic organisms was taken in from water or through gills or body surface.

Half-life in body obtained from the indoor test on the excretion rate of nonylphenol taken into the body was reported at 0.55 days in carp. This value was similar to 19-20 hours (0.8 days) in rainbow trout²⁴⁾ and 0.41 days in red medaka²⁰⁾.

Based on the data mentioned above, it was supposed that nonylphenol in the environment would be taken into the body of fish mainly from water through gills and body surface, and that the concentration of nonylphenol in fish body would be several hundred times higher than that in the water at the maximum, but it would be excreted outside the body in a few days if the concentration in the water fell.

6. Survey results on nonylphenol concentrations in the environment

(1) Results of environmental surveys in Japan

Concentrations of nonylphenol in the water, bottom sediment, soil, aquatic organism and biota at a total of 2,330 sites all over the country were measured in the Nationwide Urgent Endocrine Disrupters Survey (1998) and the Nationwide Exogenous Endocrine Disrupters Survey (1999) conducted by the Environment Agency, as well as in the Endocrine Disrupters Survey at Public Water Areas (1998) and the Endocrine Disrupters Survey at Public Water Areas (1999) conducted by the Ministry of Construction.

According to the results (Table 2), in the water quality survey, nonylphenol was detected at 617 sites out of total 1,574 sites in two years (detection ratio: 39 %), and its concentration range was NDs (<0.03-0.1) - 21 [g/L. The arithmetic mean concentration was 0.17 [g/L (calculated assuming NDs = 0), and median, 75th percentile(*), 90th percentile(*) and 95th percentile(*) concentrations were respectively ND, 0.10 [g/L, 0.30 [g/L and 0.59 [g/L. The arithmetic mean concentration was 0.19 [g/L and 0.22 [g/L if calculated based on the assumption that NDs are half of the detection limit values and are equal to them respectively. The distribution curve of the numbers of detected samples by detected concentration, as shown in Fig.4, indicated that there were two groups of different characters with roughly 95 percentile as a demarcation line.

In the bottom sediment survey, nonylphenol was detected at 146 samples out of total 293 samples in two years (detection rate: 50 %), and its concentration range was NDs (<3-87) - 12,000 [g/kg. The

arithmetic mean concentration was 282 [g/kg (calculated assuming NDs = 0), and median, 75th percentile, 90th percentile and 95th percentile concentrations were ND, 79 [g/kg, 470 [g/kg and 2,000[g/kg, respectively. Arithmetic mean concentration was 293 [g/kg and 304 [g/kg if calculated based on the assumption that NDs are half of the detection limit values and are equal to them, respectively.

In the aquatic organism survey, nonylphenol was detected at 42 samples out of total 141 samples (detection ratio: 30 %). Its concentration range was NDs (<15) - 780 [g/kg, and arithmetic mean concentration was 23[g/kg (assuming NDs = 0). Median, 75th percentile, 90th percentile and 95th percentile concentrations were ND, 17[g/kg, 42[g/kg and 99 [g/kg, respectively. The arithmetic mean concentration was 29[g/kg and 34 [g/kg based on the assumption that NDs are half of the detection limit values and are 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.

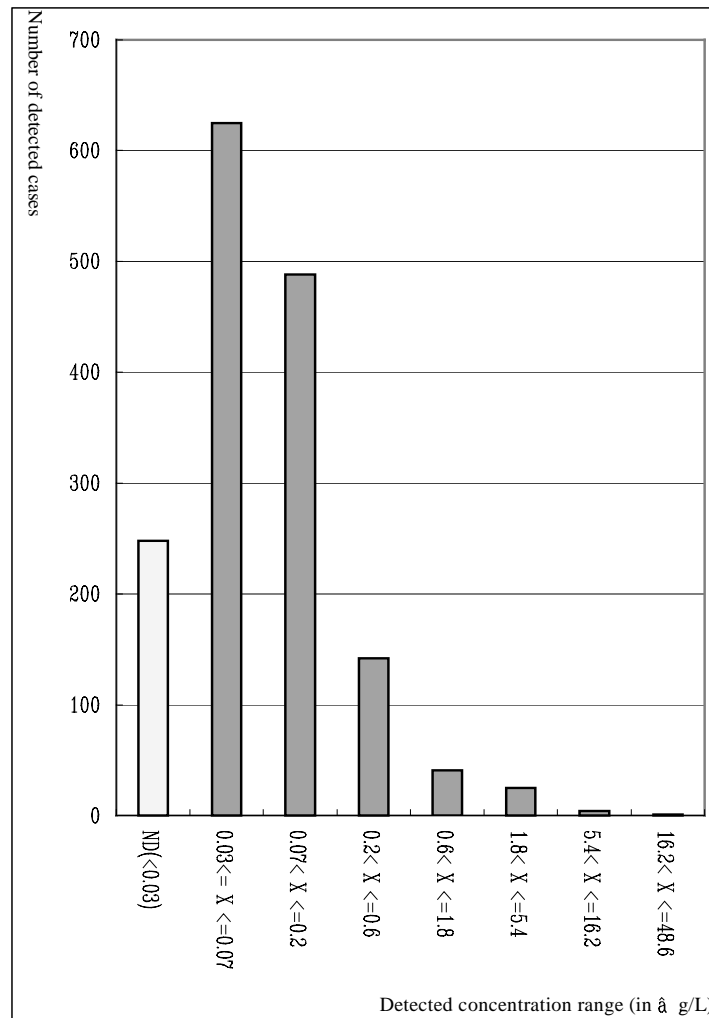


Fig. 4 Distribution of detected samples by detected concentration

N.B.: Detected concentration range was distributed by a common ratio of 3, centering around 0.6 [g/L. For the samples that are NDs, detected concentrations lower than 0.1 [g/L were distributed evenly for convenience.

Table 2 Results of the surveys on nonylphenol concentrations in the environment

Survey categories	State of detection	Concentration range	Mean concentration			75th percentile	90th percentile	95th percentile
			NDs = 0	NDs = 1/2	NDs = Detection limit values			
Water quality (g/L)	617/1,574	NDs(<0.03 ±0.1 ±21	0.17	0.19	0.22	0.10	0.30	0.59
Sediments (g/kg)	146/293	NDs (<3±87) ±12,000	282	293	304	79	470	2,000
Aquatic organisms (g/kg)	42/141	NDs(<15)±780	23	29	34	17	42	99

(2) Environmental concentrations outside Japan

According to the report by Environment Canada/Health Canada¹⁷⁾, in the surveys at Canadian rivers and lakes (42 sites, 126 samples) conducted by Environment Canada, concentrations of nonylphenol ranged from NDs (<0.02) to 4.25 g/L, averaging 0.20 g/L in river water, and from NDs (<0.02) to 0.06 g/L in lake water. Concentrations in untreated effluents from textile mills were in the range of 2.68-13.3 g/L, and those in-situ-treated effluents were 0.09-3.56 g/L. Concentrations in effluents released from textile mills to local municipal wastewater treatment plants were 0.23-25.6 g/L. Concentrations in effluents from paper mills were NDs (<0.02)-26.2 g/L before 1998, but decreased to NDs (<0.10)-4.3 g/L after 1998 on. This was reportedly the result of reduced use of nonylphenol ethoxylates in paper manufacturing process. Concentrations in the final effluents from local municipal wastewater treatment plants were NDs (<0.02)-62.1 g/L after primary treatment, 0.12-4.79 g/L after secondary treatment, and NDs (<0.02)-3.20 g/L after tertiary treatment. Concentrations in untreated sewage were 0.69-156 g/L.

According to the report by the European Commission⁷⁾, concentrations in river water in Switzerland were NDs (0.3)-45 g/L before 1996, but decreased to NDs-0.3 g/L after 1998 on. Concentrations in river water in the United Kingdom were in the range of NDs (<0.2)-180 g/L. From various survey results, the European Commission estimated the background concentration of nonylphenol in surface water in Europe at 0.2 g/L.

Further, concentrations of nonylphenol were 10-4,000 g/L in effluents from car washes, 21 g/L in sewage, 6.7 g/L (in the U.K.) in effluents from wastewater treatment plants after primary treatment, and 13-63 g/L (in Switzerland) and 0.2-2.9 g/L (in the U.K.) in effluents from wastewater treatment plants after secondary treatment.

In the surveys covering 30 rivers in the U.S.A. conducted in 1992, concentrations of nonylphenol were in the range of NDs (<0.11)-0.64 g/L, averaging 0.12 g/L. Concentrations in effluents from the wastewater treatment plants were 1-5 g/L.

7. General toxicity

(1) Acute toxicity

Stated below are the reports on acute toxicity as reported by the European Commission or Environment Canada/Health Canada. Reliability assessment has not been made by MoE.

(a) Fish

- The European Commission reported acute toxicity tests by Brooke^{27,30}, Holcombe et al³¹, and Ward and Boeri³² using fathead minnow, bluegill, rainbow trout, or sheepshead minnow as test organisms⁷. As a result, 96-hour median lethal concentrations (LC_{50s}) were in the range of 128 to 310 [g/L, and effect at the lowest concentration was observed in fathead minnow (*Pimephales promelas*)³⁰. The smallest Lowest-Observed-Effect Concentration (LOEC) value was 98 [g/L in the test for loss of equilibrium sense using nonylphenol (purity 91%, kind of isomer unknown) as test substance and fathead minnow as test organism³¹. The smallest No-Observed-Effect Concentration (NOEC) value was 83.1 [g/L in the test for survival using nonylphenol (kind of isomer unknown) as test substance and fathead minnow as test organism²⁷.
- Environment Canada/Health Canada reported that the median lethal concentrations (LC_{50s}) for 18 species of fish ranged from 17 to 1400 [g/L, mostly in 100-300 [g/L¹⁷.

(b) Aquatic invertebrates

- The European Commission reported acute toxicity tests by Ward and Boeri³³, Brooke³⁰, England³⁴, England and Bussard³⁵, Huls³⁶, and Comber et al³⁷ using daphnids, mysids, amphipods, lobworms, snails or dragonflies as test organisms⁷. As a result, 96-hour LC_{50s} ranged from 43 to 774 [g/L, and effect at the lowest concentration was observed in mysids (*Mysidopsis bahia*)³³. The smallest NOEC value was 18 [g/L in the test using 4-nonylphenol (branched) as test substance and mysids as test organisms³³.
- Environment Canada/Health Canada reported LC_{50s} for aquatic invertebrates ranging from 20 to 3000 [g/L¹⁷.

(c) Algae

- The European Commission reported acute toxicity tests by Kopf³⁸, Ward and Boeri^{39,40}, Huls⁴¹, and Brooke³⁰ using three species of phytoplankton as well as duckweed as test organisms⁷. As a result, 72-hour or 96-hour median effective concentrations (EC_{50s}) were in the range of 27 to 1,300 [g/L, and effect at the lowest concentration was observed in marine diatoms (*Skeletonema costatum*)³⁹. The smallest NOEC value adopted was the 72-hour 10% effective concentration (EC₁₀) 3.3 [g/L as reported in the test for reproduction using nonylphenol (kind of isomer unknown) as test substance and freshwater green alga (*Scenedesmus subspicatus*) as test organism³⁸.
- Environment Canada/Health Canada reported LC_{50s} for algae ranging from 27 to 2500 [g/L¹⁷.

(2) Chronic toxicity

Stated below are the reports on chronic toxicity as reported by the European Commission and Environment Canada/Health Canada. Reliability assessment has not been made by MoE

(a) Fish

- The European Commission reported chronic toxicity tests by Ward and Boeri⁴² and Brooke²⁷ using fathead minnow as test organism for 28 or 33 days⁷. As a result, the smallest LOEC value was 14 [g/L in the test for survival using 4-nonylphenol (branched) as test substance and fathead minnow as test organism⁴². The smallest NOEC value was 7.4 [g/L in the test for survival using 4-nonylphenol (branched) as test substance⁴².
- Environment Canada/Health Canada reported 6 [g/L as NOEC for fish¹⁷.

(b) Aquatic invertebrates

- The European Commission reported chronic toxicity tests by Ward and Boeri⁴³, Comber et al³⁷, England³⁴, and England and Bussard⁴⁴ using mysids, daphnids or midges as test organ-

isms for 7 to 28 days⁷⁾. As a result, LC₅₀s ranged from 100 to 258 [g/L, and effects at the lowest concentration was observed in *Daphnia magna*³⁷⁾. The smallest LOEC was 6.7 [g/L in the test for growth using 4-nonylphenol (branched) as test substance and mysids as test organisms⁴³⁾. The smallest NOEC was 3.9 [g/L in the test for growth using 4-nonylphenol (branched) as test substance and mysids as test organisms⁴³⁾.

- Environment Canada/Health Canada reported 3.9 [g/L as NOEC for aquatic invertebrates¹⁷⁾.

(3) Reproductive toxicity

(a) Fish

The reports on reproductive toxicity to fish obtained through JICST are stated below. Reliability assessment are not made by MoE.

- Shioda and Wakabayashi⁴⁵⁾ studied effects on male and female medaka (*Oryzias latipes*) exposed to 4-nonylphenol (mixture of 90% p-NP and 10% o-NP, branched) at 6.6, 22 or 66 [g/L (set values) for 2 weeks. As a result, significant decrease was observed in the number of eggs produced when females in the group exposed to 6.6 [g/L or higher were mated with males not exposed.

(b) Aquatic invertebrates

The reports on reproductive toxicity to aquatic invertebrates as reported by the European Commission are stated below. Reliability assessment has not been made by MoE.

- The European Commission reported reproductive toxicity tests for 7 or 21 days by England³⁴⁾ and Huls^{46,47)} using daphnids as test organisms⁷⁾. As a result, the smallest LOEC was 140 [g/L as obtained in the test using nonylphenol (isomer mixture) as test substance and *Daphnia magna* as test organism⁴⁷⁾. The smallest NOEC was 88.7 [g/L in the test using 4-nonylphenol (branched) as test substance and *Ceriodaphnia dubia* as test organism³⁴⁾.

8. Literature search and reliability assessment on suspected endocrine disrupting effects on fish, etc.

(1) Effects on fish

(a) *In vitro* tests

Stated below are the results of *in vitro* tests on endocrine disrupting effects on fish as reported in the literature of 1972-2000 obtained through TOXLINE, etc. and as reported by the European Commission or Environment Canada/Health Canada. Reliability assessment has not been made by MoE.

It is noted that *in vitro* test reports on estrogenic effects due to nonylphenol were found, but that there were no reports on androgenic effects.

- Loomis and Thomas⁴⁸⁾ tested binding affinity of estrogenic receptors derived from testes and livers of Atlantic croakers in 4-nonylphenol (97%, 4-NP, branched, according to manufacturer) at concentrations of 10⁻⁶ to 10⁻⁴ M. As a result, estrogenic receptors from testes showed binding to 4-nonylphenol at EC₅₀ of 1.3 · 10⁻⁶ M. Their affinity was about 1/3000, as compared with 17 β-estradiol. Estrogenic receptors from livers showed binding to 4-nonylphenol at EC₅₀ of 1.5 · 10⁻⁵ M. Affinity was about 1/2000, as compared with 17 β-estradiol.
- White et al.⁴⁹⁾ studied the responses of primary cultures of male rainbow trout hepatocytes exposed to 4-nonylphenol (4-NP, branched, according to the authors) at concentrations of 10⁻⁷, 10⁻⁶ or 10⁻⁵ M, and the quantity of binding/displacement between estrogen receptor from hepatocytes and 17 β-estradiol. As a result, vitellogenin expression was observed in cultured hepatocytes at concentrations of 10⁻⁶ M or higher, and receptor binding inhibition was observed at K_d of 5 · 10⁻⁵ M.
- Milligan et al.⁵⁰⁾ studied the quantity of binding/displacement between sex steroid binding pro-

tein in rainbow trout blood plasma and 17 β -estradiol at the concentrations of 4-nonylphenol (technical grade; 97%, 4-NP, branched, according to manufacturer's catalog) ranging from 10^{-6} to $3 \cdot 10^{-3}$ M. As a result, binding was observed, and activity was 1/10,000 or lower, as compared with that of 17 β -estradiol.

- Fluoriot et al. ⁵¹⁾ studied the responses of primary cultures of male rainbow trout hepatocytes exposed to nonylphenol (kind of isomer unknown) at concentration of 10^{-7} M for 24 hours. As a result, expression of vitellogenin mRNA and estrogen receptor mRNA were observed in cultured hepatocytes. Activity ranged from 1/5000 to 1/500, as compared with that of 17 β -estradiol.
- Islinger et al. ⁵²⁾ studied the responses of primary cultures of rainbow trout hepatocytes exposed to technical nonylphenol (4-NP, branched, according to manufacturer's catalog) at concentrations of 10^{-6} , 10^{-5} or 10^{-4} M for 96 hours. As a result, expression of vitellogenin mRNA was observed in cultured hepatocytes at 10^{-6} M or higher. Activity ranged from 1/3000 to 1/2000, as compared with that of 17 β -estradiol.
- Celius et al. ⁵³⁾ studied the responses of primary cultures of Atlantic salmon hepatocytes exposed to 4-nonylphenol (purity 85%, *p*-isomer mixture; 4-NP, branched, according to manufacturer's catalog) at concentrations of 1, 5 or 10 μ M. As a result, expression of egg membrane proteins (zona radiata proteins) was observed in cultured hepatocytes exposed at 5 μ M or higher for 48 hours, and expression of vitellogenin was observed in cultured hepatocytes exposed at 10 μ M or higher for 96 hours
- Jobling and Sumpter ⁵⁴⁾ studied the responses of primary cultures of male rainbow trout hepatocytes exposed to 4-nonylphenol (4-NP, branched, according to the authors) at concentrations of 1, 10, 50 or 100 μ M for 2 or 4 days. As a result, vitellogenin released from cultured hepatocytes to culture medium was observed at 10 μ M or higher, and ED₅₀ was 16.15 μ M. Activity was about 1/111,000, as compared with that of 17 β -estradiol.
- Arukwe et al. ⁵⁵⁾ studied the responses of liver granules of Pacific salmon intraperitoneally administered 4-nonylphenol (purity 85%, isomer mixture) at concentrations of 1, 5, 25 or 125 mg/kg. As a result, 6 β -hydroxylation enzyme activity in granules increased in the group administered 1 mg/kg, and decreased in the group administered 25 mg/kg or more. 16 α - and 17 α -hydroxylation enzyme activity and 7-ethoxyresorufin-o-deethylase activity decreased in the group administered 125 mg/kg. CYP1A protein decreased depending on dose in the group administered 1 mg/kg or more, and CYP2K-like and CYP3A-like proteins decreased in the group administered 125 mg/kg. It was concluded that 4-nonylphenol would increase steroid metabolising enzymes at low concentrations and decrease them at high concentrations.

(b) Animal tests (*in vivo* tests)

Of the literature information of 1972-2000 obtained through TOXLINE, etc., the following reports are found to show suspected endocrine disrupting effects to fish and observed such effects. whose reliability was acknowledged in the reliability assessment conducted by MoE.

- Miles-Richardson et al. ⁵⁶⁾ studied effects on male and female fathead minnows exposed to 4-*p*-nonylphenol (4-NP, branched, according to manufacturer) at concentrations of 0.05, 0.16, 0.4, 1.6 or 3.4 μ g/L (measured values) for 42 days. As a result, abnormality in testis tissue was observed, among males in the group exposed to 1.6 μ g/L or higher, in the tissue examination using electron microscope.
- Ren et al. ⁵⁷⁾ studied effects on juvenile rainbow trout exposed to nonylphenol (kind of isomer unknown) at concentrations of 10, 20, 50, 100 or 150 μ g/L (sets values) for 72 hours. As a result, in the group exposed to 10 μ g/L or higher, expression of vitellogenin mRNA was observed in liver.
- Jobling et al. ⁵⁸⁾ studied effects on mature male rainbow trout exposed to 4-nonylphenol (4-NP, branched, according to the authors) at concentrations of 0.24, 1.06, 1.85, 5.02, 20.3 or 54.3

µg/L (measured values) for 3 weeks. As a result, in the group exposed to 20.3 µg/L or higher, induction of vitellogenin was observed in plasma. Threshold value of vitellogenin induction was supposed to be 10 µg/L.

- Gray and Metcalfe⁵⁹⁾ studied effects on male medaka (*Oryzias latipes*) exposed to tech-4-nonylphenol at 10, 50 or 100 µg/L for 3 months. As a result, in the group exposed to 50 µg/L or higher, formation of egg cells was observed in testes.
- Pedersen et al⁶⁰⁾ studied effects on juvenile rainbow trout exposed to tech-nonylphenol (4-NP, branched, purity 90%) at a concentration of 76 µg/L (measured value) for 9 days. As a result, increase in vitellogenin concentration was observed in plasma. Further, effects on juvenile rainbow trout intraperitoneally administered tech-nonylphenol or 4-*n*-nonylphenol twice at a concentration of 50 mg/kg were studied. As a result, a significant vitellogenin induction in plasma was observed in the tech-nonylphenol administered group, but not in the 4-*n*-nonylphenol administered group.
- Korsgaard and Pedersen⁶¹⁾ studied effects on zoarcidae exposed to tech-4-*t*-nonylphenol (branched) at concentrations of 10, 50, 100, 250, 500 or 1,000 µg/L (set values) for 3 weeks. As a result, in the group exposed to 100 µg/L or higher, increase in vitellogenin concentration was observed in plasma.

The contents reported by European Commission or Environment Canada/Health Canada are stated below. Reliability assessment has not been made by MoE.

- Fent et al.⁶²⁾ studied effects on juvenile rainbow trout exposed to nonylphenol (kind of isomer unknown) at concentrations of 1 or 10 µg/L for 12 months starting from the egg stage in the 20th day of fertilization. As a result, increase in the quantities of vitellogenin mRNA and vitellogenin was observed in livers of male rainbow trout exposed to 1 or 10 µg/L. This report is cited by Environment Canada/Health Canada¹⁷⁾, but not adopted by the European Commission, because it is a lecture summary and details are unknown.
- According to the report by the European Commission⁷⁾, Ashfield et al⁶³⁾ studied effects on hereditary total female rainbow trout exposed to 4-*tertiary*-nonylphenol (branched) at concentrations of 1, 10 or 30 µg/L for 35 days. As a result, increase in relative ovary weight to body weight was observed in the group exposed to 30 µg/L.
- According to the report by the European Commission⁷⁾, Christensen et al⁶⁴⁾ studied effects on male right eye flounders intraperitoneally administered nonylphenol for 2 weeks. As a result, vitellogenin was observed in plasma in the group administered 10 mg/kg.
- According to the report by the European Commission⁷⁾, Nimrod and Benson⁶⁵⁾ studied effects on catfish administered nonylphenol intraperitoneally. As a result, a significant increase in vitellogenin was observed in plasma in the group administered nonylphenol 237 mg/kg. Activity was 1/5,000, as compared with that of 17β-estradiol.

(c) Field-survey results in Japan

Stated below is the literature information on field surveys in Japan as obtained through JICST. Reliability assessment is not made by MoE.

- Nakamura and Iguchi⁶⁶⁾ made histological examination of carp gonad, etc., vitellogenin assay, and measurement of nonylphenol, etc. in river water, in their field surveys on carps living in the areas of the Tama River where treated wastewater joins mainstream water between, wastewater treatment plant and the mainstream. In the past five surveys, 66 females, 38 males and 1 hermaphroditic carp were examined. Their body length was in the range of about 45-65 cm, and estimated age in 6-9 years. About 30% of males had abnormally small testes and extremely poor spermatogenesis. Expression of vitellogenin was observed in about 57% of males. Nonylphenol concentrations in river water were 0.25 µg/L and 0.47 µg/L at the places where the carps were collected. Causes for the abnormality are still unknown.

(2) Effects on other aquatic organisms

Of the literature information of 1972-2000 obtained through TOXLINE, etc., the following reports which showed suspected endocrine disrupting effects to aquatic organisms other than fish and observed such effects were found. Their reliability was acknowledged in the reliability assessment carried out by MoE.

(a) Animal tests (*in vivo* tests)

- Kahl et al.⁶⁷⁾ studied effects on egg lumps of the midges exposed to 4-nonylphenol (4-NP, branched, according to manufacturer) at concentrations of 8, 18, 36, 84 or 138 [g/L (measured values) for 20 days. As a result, morphogenic abnormality was observed in the group exposed to 36 [g/L or more.

9. Screening, tests, etc. using fish

(1) *In vitro* assays

(a) Competitive binding assay to medaka estrogen receptor α (ER α)

Competitive receptor binding assay was performed using medaka (*Oryzias latipes*) and human estrogen receptor α ligand binding domains expressed in *E. coli*., and the binding affinities of nonylphenol (mixture), 4-*t*-octylphenol, 4-*t*-pentylphenol and 4-*t*-butylphenol to these recombinant receptors were measured. Release of the radiolabelled ligand from medaka ER α depending on the concentrations of 17 β -estradiol, nonylphenol, and 4-*t*-octylphenol were observed (Fig. 5). Their relative binding affinities (RBA) to both medaka and human receptors compared with 17 β -estradiol were summarized in table 3. It was found that alkylphenols with branched alkyl chain bound to medaka ER α according to their chain length and RBA values were about several hundreds times stronger than those to human ER α . Especially, nonylphenol (mixture) and 4-*t*-octylphenol had high receptor binding abilities and their RBA values were about 1/10 and 1/15 of 17 β -estradiol, respectively. Other branched alkylphenols tested also showed relatively high receptor binding abilities. The RBA values of 4-*t*-pentylphenol and 4-*t*-butylphenol were 1.1 and 0.15, respectively, and they were hundreds times greater than those to human ER α as in the case with nonylphenol and 4-*t*-octylphenol. On the other hand, linear alkylphenols bound to medaka ER α weakly. Their RBA values were less than 0.1% when compared with 17 β -estradiol and almost alike to those to human ER α .

Furthermore, the binding abilities of nonylphenol were examined for medaka ER β , and ERs α from other fish, carp (*Cyprinus carpio*) and mummichog (*Fundulus heteroclitus*) with a same procedure. The RBA values of nonylphenol to medaka ER β and mummichog ER α were 1/110 and 1/200 of 17 β -estradiol, respectively. However, it bound to carp ER α weaker than to other fish ERs (RBA \sim 0.1%). In conclusion, alkylphenols with branched bulky alkyl chains showed relatively high binding affinities to estrogen receptors from fish compared with those to human estrogen receptor.

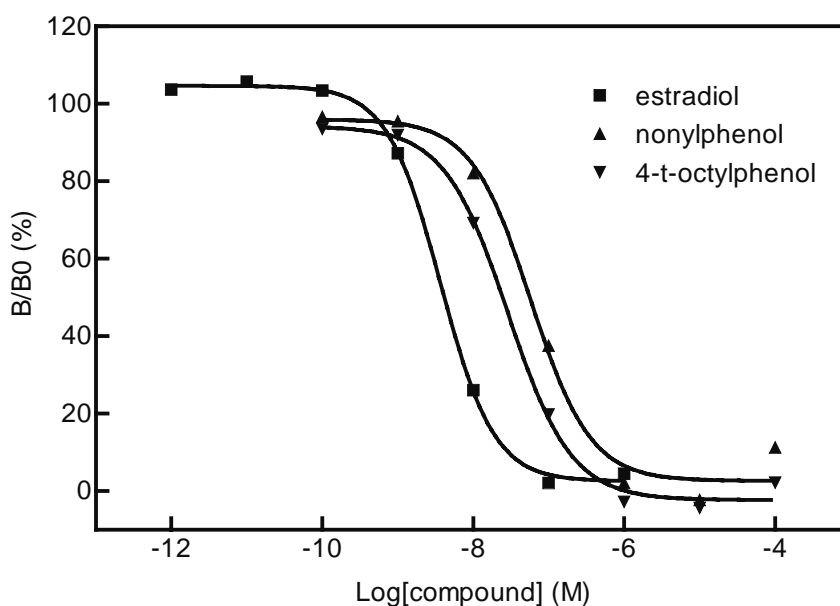


Fig. 5 Dose-response curves of 17β -estradiol and alkylphenols in the radioligand receptor binding assay using [^3H] 17β -estradiol and medaka ER α expressed in *E. coli*.

Table 3 IC_{50} values and relative binding affinities (%) of alkylphenols to medaka and human estrogen receptors α ligand binding domain

Chemical substances	Medaka #1		Human #2	
	IC_{50} values (M)	Relative binding affinity (%)	IC_{50} values (M)	Relative binding affinity (%)
Estradiol	4.8×10^{-9}	100	2.1×10^{-9}	100
Nonylphenol (mixture)	7.9×10^{-8}	8.1	3.4×10^{-6}	0.061
4- <i>t</i> -Octylphenol	3.2×10^{-8}	16	6.6×10^{-6}	0.032
4- <i>t</i> -Pentylphenol	3.9×10^{-7}	1.1	4.1×10^{-5}	0.0051
4- <i>t</i> -Butylphenol	3.0×10^{-6}	0.15	1.6×10^{-4}	0.0013
4- <i>n</i> -Nonylphenol	1.1×10^{-6}	0.038	4.2×10^{-6}	0.050
4- <i>n</i> -Octylphenol	5.3×10^{-6}	0.077	1.1×10^{-5}	0.020
4- <i>n</i> -Pentylphenol	5.5×10^{-6}	0.084		
4- <i>n</i> -Butylphenol	6.5×10^{-6}	0.066	8.8×10^{-5}	0.0024

#1: Measured four times for nonylphenol (mixture) and 4-*t*-octylphenol, and three times for other chemical substances.

#2: Measured three times for all chemical substances.

(b) Reporter gene assay

Transcriptional activities of alkylphenols mediated by medaka ER α were measured using HeLa cells transiently co-transfected with both receptor expression and reporter (firefly luciferase) vectors. It was found that transactivation function presented by EC50 value of nonylphenol was three hundred times weaker than 17β -estradiol (Fig. 6).

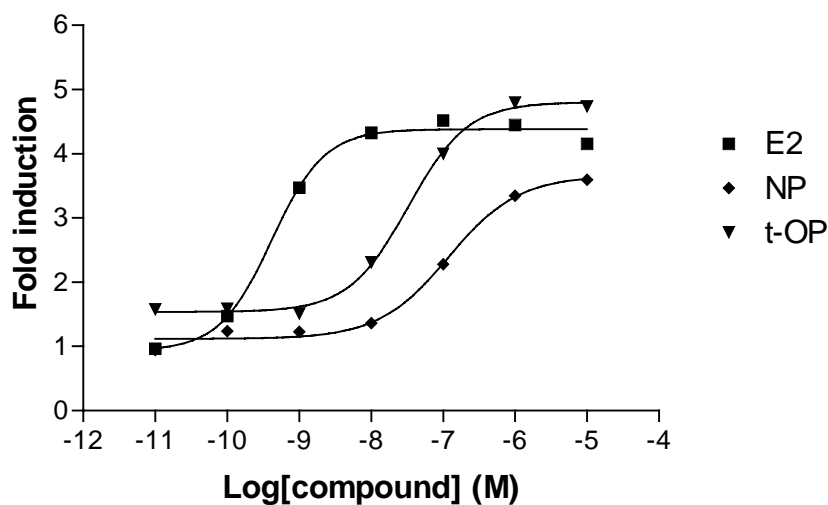


Fig. 6 Reporter gene transactivation assay using HeLa cells co-transfected with medaka estrogen receptor expression and reporter vectors.

(2) *In vivo* studies using medaka

(a) Screening

(i) Medaka vitellogenin assay

The estrogenic potency of nonylphenol (mixture; NP) and 4-*t*-octylphenol (4-*t*-OP) was evaluated using *in vivo* vitellogenin (precursor of egg yoke protein) synthesis in medaka. About 3-month-old medaka (respectively 8 females and males / treatment) were exposed to 5 test concentrations of each substance (NP; 7.40, 12.8, 22.5, 56.2 and 118 [g/L, 4-*t*-OP; 12.7, 27.8, 64.1, 129 and 296 [g/L as 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 exposed fish were removed, and vitellogenin concentration in each liver was measured.

In either NP or 4-*t*-OP study, any death or particular symptom was not observed through the exposure period. The hepatic vitellogenin concentrations in males were increased in a concentration-dependent manner, and a statistically significant induction was observed at ≥ 22.5 [g/L for NP study and ≥ 64.1 [g/L for 4-*t*-OP study (Fig.7).

These results suggested that both NP and 4-*t*-OP could cause vitellogenin synthesis in the livers of male medaka through their estrogenic activities.

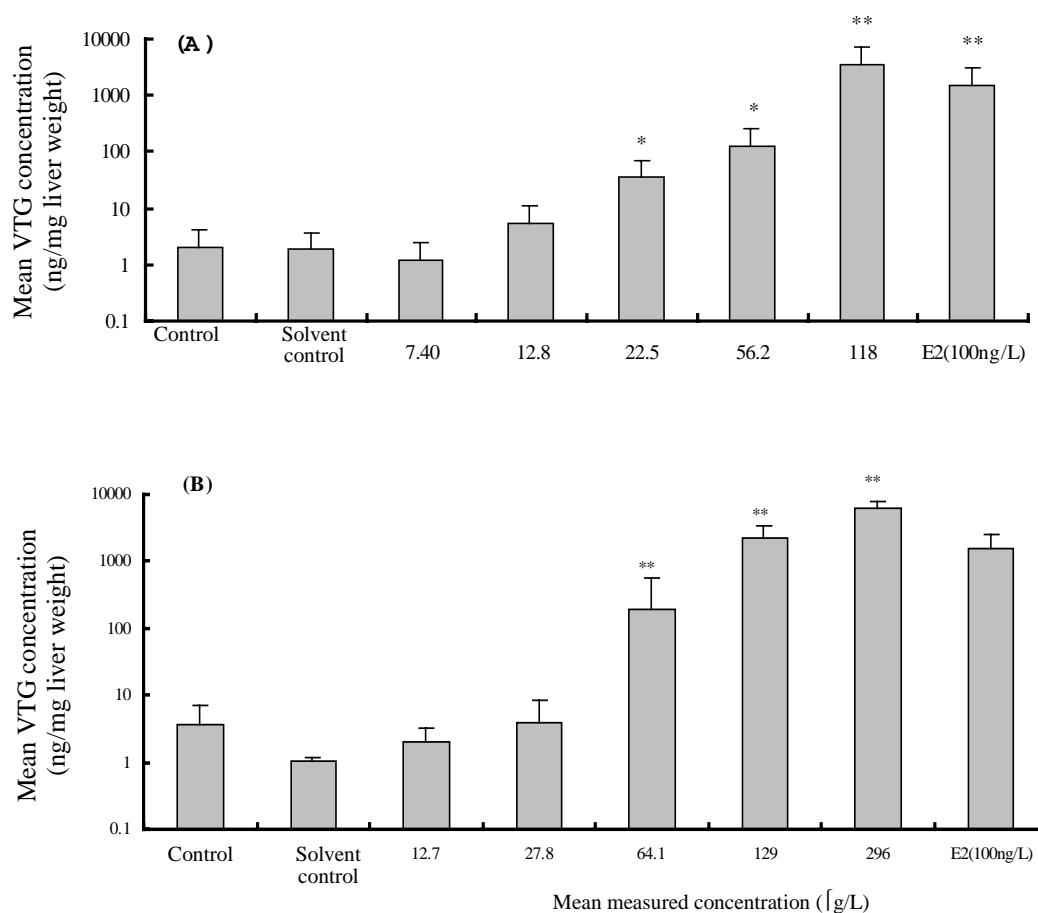


Fig. 7 Vitellogenin (VTG) concentrations in the livers of male medaka (*Oryzias latipes*) in NP study (A) and 4-*t*-OP study (B). Data is shown as mean±standard deviation. * and ** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

(ii) Medaka partial life test

This test was performed to assess endocrine disrupting effects of nonylphenol (mixture; NP) and 4-*t*-octylphenol (4-*t*-OP) on sex differentiation of medaka. Medaka (60 eggs/treatment) were exposed to 5 test concentrations of each substance (NP; 3.30, 6.08, 11.6, 23.5 and 44.7 [g/L, 4-*t*-OP; 6.94, 11.4, 23.7, 48.1 and 94.0 [g/L as mean measured concentrations) under flow-through conditions from fertilized eggs to 60-day posthatch. During the exposure period, hatching, posthatch mortality, and abnormal behavior and appearance were observed daily. At the end of exposure (at 60-day posthatch), the total length and body weight of all the surviving fish were measured, and the sex of each individual was determined from the appearance of secondary sex characteristics. Furthermore, 20 individuals from each treatment group were randomly sampled, and then their livers and gonads were removed for vitellogenin measurement and gonadal histology.

In either NP or 4-*t*-OP test, any particular effect on hatching of fertilized eggs and posthatch mortality was not observed at the concentrations tested. As for growth of fish at 60-day posthatch in the NP test, however, a significant decrease was observed in both total length and body weight in the 44.7 [g/L treatment, and in body weight in the 23.5 [g/L treatment. This result suggests that NP adversely affects the growth of medaka. In the 4-*t*-OP test, no

growth reduction was observed at the concentrations tested. The sex ratio estimated from the appearance of secondary sex characteristics of the surviving fish at 60-day posthatch was significantly skewed toward female at ≥ 23.5 $\mu\text{g/L}$ in NP test and ≥ 48.1 $\mu\text{g/L}$ in 4-*t*-OP test (Tables 4 and 5). Furthermore, gonadal histology showed that the fish in ≥ 11.6 $\mu\text{g/L}$ NP treatment groups and ≥ 11.4 $\mu\text{g/L}$ 4-*t*-OP treatment groups had testis-ova as shown by the presence of oocytes in the testis (hereinafter referred to as testis-ova)(Tables 4 and 5). The hepatic vitellogenin concentrations in males exposed to ≥ 11.6 $\mu\text{g/L}$ NP and ≥ 11.4 $\mu\text{g/L}$ 4-*t*-OP were significantly increased(Fig.8).

These results indicate that both NP and 4-*t*-OP exert estrogenic effects on sex differentiation of male medaka, and suggest that the Lowest-Observed-Effect Concentrations (LOECs) of NP and 4-*t*-OP for feminization of the appearance of their secondary sex characteristics were 23.5 $\mu\text{g/L}$ and 48.1 $\mu\text{g/L}$, respectively, and that the LOECs of them for induction of testis-ova and vitellogenin were 11.6 $\mu\text{g/L}$ and 11.4 $\mu\text{g/L}$.

Table 4 Sex ratios as determined by gross examination of secondary sex characteristics of medaka (*Oryzias latipes*) at 60-day posthatch in NP test, and by their gonadal histology.

NP concentration ($\mu\text{g/L}$)	Secondary sex characteristics		Gonadal histology			
	N: Number of fish	Sex ratio (B: \geq)	N: Number of fish			
			Testis	Ovary	Testis-ova	
Control	55	25 : 30	20	8	12	0
Solvent control	57	27 : 30	20	10	10	0
3.30	59	27 : 32	20	9	11	0
6.08	59	25 : 34	20	10	10	0
11.6	57	28 : 29	20	9	7	4*
23.5	58	11 : 47**	20	2	9	9**
44.7	60	1 : 59**	20	1	15	4**

* and ** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

Table 5 Sex ratios as determined by gross examination of secondary sex characteristics of medaka (*Oryzias latipes*) at 60-day posthatch in 4-*t*-OP test, and by their gonadal histology.

4- <i>t</i> -OP concentration ($\mu\text{g/L}$)	Secondary sex characteristics		Gonadal histology			
	N: Number of fish	Sex ratio(B: \geq)	N: Number of fish			
			Testis	Ovary	Testis-ova	
Control	55	25 : 30	20	10	10	0
Solvent control	56	21 : 35	20	9	11	0
6.94	55	26 : 29	20	10	10	0
11.4	56	25 : 31	20	8	11	1
23.7	48	13 : 35	20	8	10	2
48.1	56	13 : 43**	20	7	10	3*
94.0	54	0 : 54**	20	1	15	5*

* and ** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

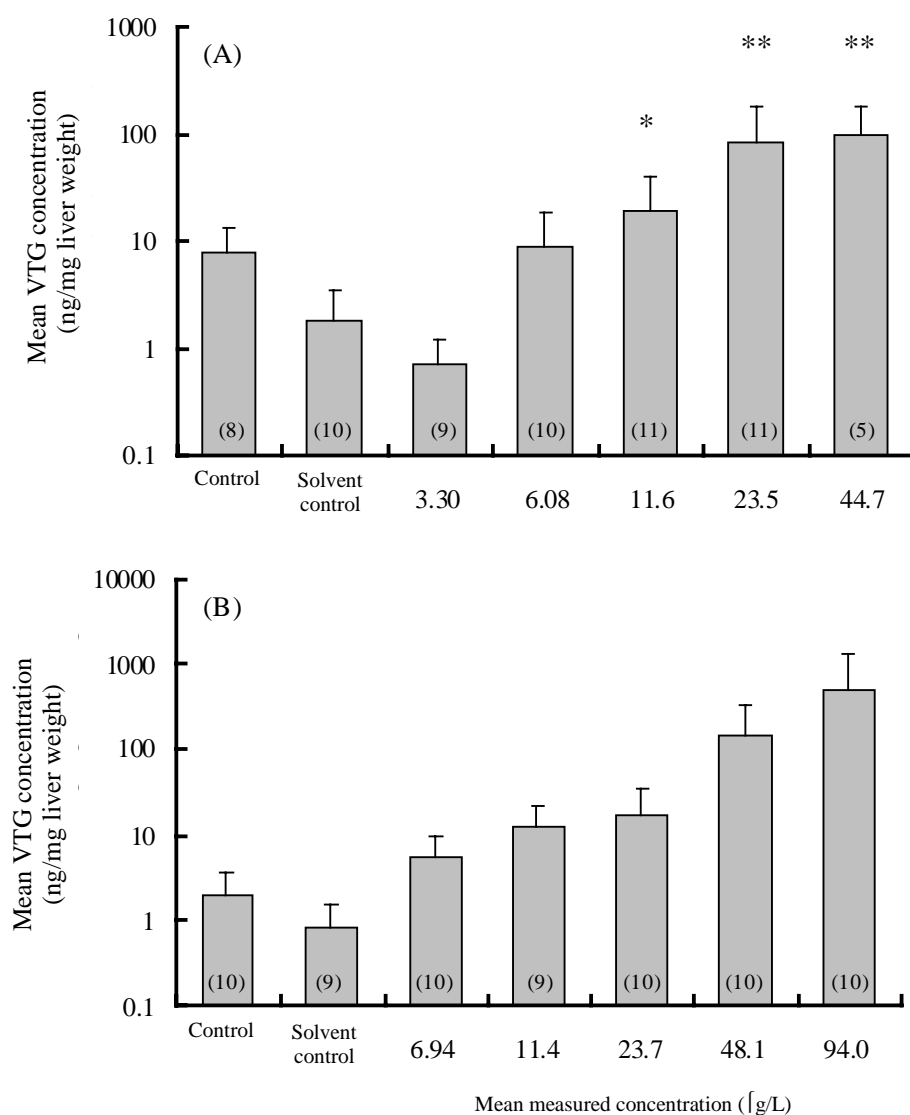


Fig. 8 Vitellogenin (VTG) concentrations in the livers of male medaka (*Oryzias latipes*) at 60-day posthatch in NP test (A) and 4-*t*-OP test (B). Data is shown as mean±standard deviation. Numbers in parentheses indicate the number of fish. * and ** denote significant differences at $p < 0.05$ and $p < 0.01$, respectively.

(b) Definitive test (medaka full life-cycle test)

This test was conducted to elucidate chronic toxicity and endocrine disrupting effects of nonylphenol (mixture; NP) on the life cycle of medaka. Medaka (60 eggs/treatment) were exposed to mean measured NP concentrations of 4.2, 8.2, 17.7, 51.5 and 183 $\mu\text{g/L}$ under flow-through conditions from fertilized eggs to 104-day posthatch. During the exposure period, hatching, posthatch mortality and abnormal behavior and appearance were observed daily. At 60-day posthatch, phenotype sex was determined from the appearance of secondary sex characteristics, and histological observation of gonad was made for 20 fish per treatment. Furthermore, at 70-day posthatch, 6 mating pairs in the 2 low treatment (4.2 and 8.2 $\mu\text{g/L}$) and the controls and solvent controls were selected. No pairs from the 51.5 $\mu\text{g/L}$ treatment and only 3 pairs from the 17.7 $\mu\text{g/L}$ treatment could be selected due to a skewed sex ratio and/or the limited number of surviving fish. The eggs spawned from each female were counted daily and assessed for viability until 104-day posthatch.

The fertilized eggs spawned on 102- and 103- day posthatch of the parental generation were also exposed in the same system until 60-day posthatch, and effects were examined.

The 183 [g/L treatment significantly reduced the embryo survival and swim-up success of the F0 fish. The cumulative mortality of the F0 fish from swim-up to 60-day posthatch were significantly increased in the 17.7 and 51.5 [g/L treatments. No concentration-related effect was observed on the growth of fish at 60-day posthatch. However, the sex ratio estimated from the appearance of their secondary sex characteristics was completely skewed toward female in the 51.5 [g/L treatment (Table 6). Additionally, gonadal histology showed that the fish in 17.7 and 51.5 [g/L treatments had testis-ova (Table 6). The sex ratio of the F0 fish in the 51.5 [g/L treatment was completely skewed toward female, subsequently the mating pairs from ≤ 17.7 [g/L treatments were selected at 70-day posthatch, and their fecundity and fertility were observed daily until 103-day posthatch. Fecundity was unaffected by any of the treatments examined. The mean fertility in the 17.7 [g/L treatment was reduced to 76% of that in the controls, although no statistically significant differences were determined (Fig.9). Overall, These results suggest that the lowest-observed-effect-concentration and no-observed-effect-concentration of NP through the life cycle of the F0 medaka were 17.7 [g/L and 8.2 [g/L, respectively. In the progeny generation (F1), No significant effects were observed on hatching, posthatch mortality, or growth at the concentrations tested (4.2 to 17.7 [g/L). However, induction of testis-ova in the gonads of the F1 fish at 60-day posthatch was observed in both the 8.2 [g/L and 17.7 [g/L (Table 7). This result suggests that NP could have significant effects on reproductive potential of the F1 medaka at lower concentrations than 17.7 [g/L.

Table 6 Sex ratios as determined by gross examination of secondary sex characteristics of the F₀ medaka (*Oryzias latipes*) at 60-day posthatch and by their gonadal histology.

NP concentration (g/L)	N Number of fish	Sex ratio (B:≡)	Gonadal histology		
			N Testis	N Ovary	N Testis-ova
Control	20	9 : 11	9	11	0
Solvent control	20	8 : 12	8	12	0
4.2	20	12 : 8	12	8	0
8.2	20	13 : 7	14	6	0
17.7	20	9 : 11	5	11	4
51.5 a	20	0 : 20	0	12	8

a: The sex ratio obtained from gonadal histology differed significantly from that of the solvent control at $p < 0.001$.

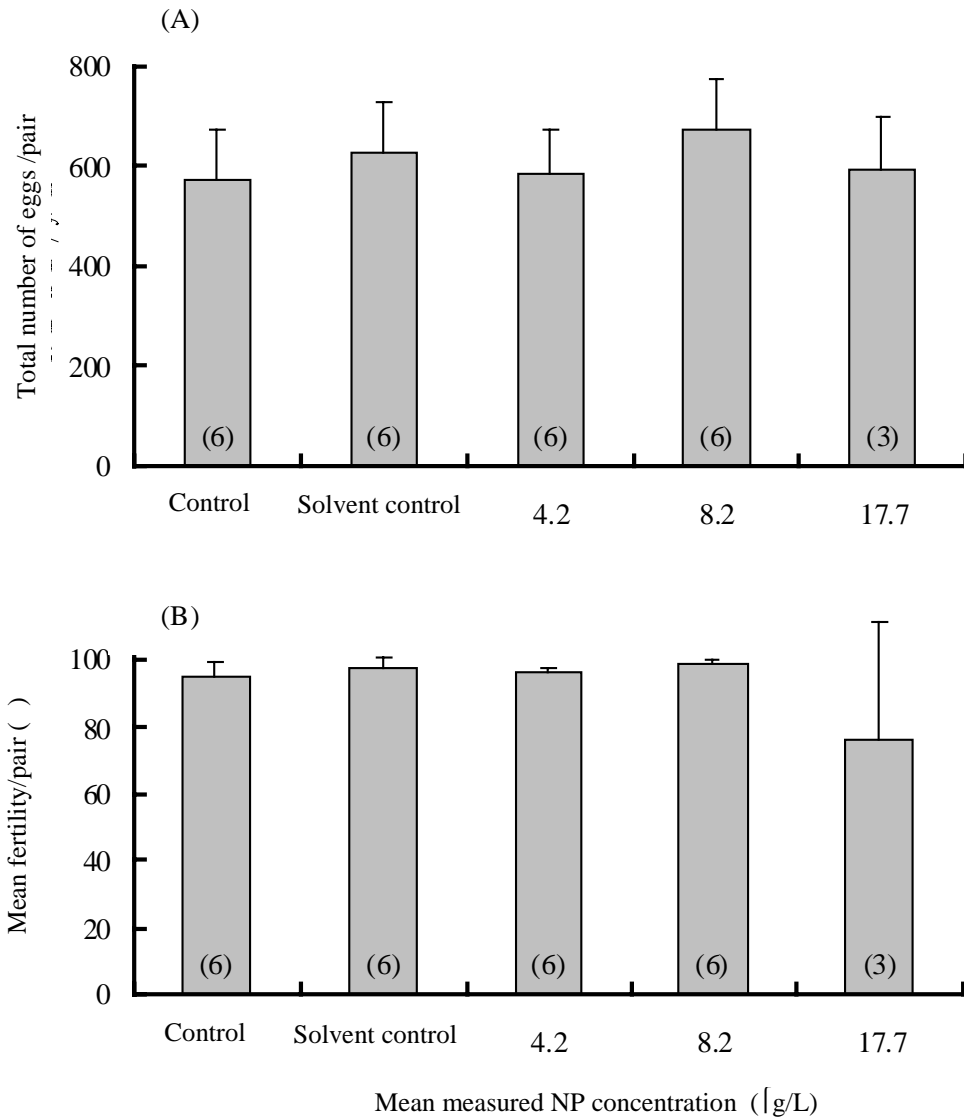


Fig. 9 Total number of eggs spawned by each pair from 71- to 104-day posthatch (A), and mean fertility per pair (B). Data is shown as mean \pm standard deviation. The number of pairs in each treatment was indicated on each bar.

Table 7 Sex ratios as determined by gross examination of secondary sex characteristics of the F₁ medaka (*Oryzias latipes*) at 60-day posthatch and by their gonadal histology.

NP concentration (µg/L)	N Number of fish	Sex ratio (B:≡)	Gonadal histology			
			N Number of fish	Testis	Ovary	Testis-ova
Control	59	28 : 31	20	7	13	0
Solvent control	54	26 : 28	20	11	9	0
4.2	54	25 : 29	20	9	11	0
8.2	49	24 : 25	20	10	8	2
17.7a	28	9 : 19	20	4	11	5

a: The sex ratio obtained from gonadal histology differed significantly at $p < 0.001$.

10. Overseas developments in risk assessment

(1) Canada

Environment Canada/Health Canada published the "Draft for public comment - Assessment Report, Nonylphenol and its Ethoxylates" in March, 2000¹⁷⁾.

The draft report covers the properties, uses, production, market trends, sources, environmental fate, environmental distribution, environmental concentrations, general toxicity, endocrine disrupting activities, bioconcentrations, etc., of nonylphenol and its ethoxylates, and makes risk assessments on human health and ecological effects in comparisons with predicted exposure levels and with predicted no-effect levels.

In its human health risk assessment, the report says that the lowest-observed-effect level is 12 mg/kg/day according to the three-generation rat study by U.S. National Toxicology Program (NTP), and that the ratio of this effect level to an predicted exposure level of 0.017 mg/kg/day via food becomes approximately 700.

In its ecological risk assessment, it says that the predicted no-effect concentration in the most conservative approach is 0.17 [g/L obtained by dividing an acute toxicity 96hLC₅₀ value of 17 [g/L in winter flounder by an assessment factor of 100 the predicted no-effect concentration in the conservative approach is 0.39 [g/L obtained by dividing the largest no-observed-effect concentration (NOEC) value of 3.9 [g/L of chronic toxicity in mysid shrimp by an assessment factor of 10, and the predicted no-effect concentration for endocrine disrupting activity is 1 [g/L obtained by dividing a threshold value of 10 [g/L of induction of vitellogenin in male rainbow trout plasma by an assessment factor of 10. Then it compares to the respective predicted no-effect concentrations and to the predicted environmental concentrations. As a result, it says concentrations in river water, factory effluents, and effluents from wastewater treatment facilities exceed predicted no-effect concentrations in some cases.

In overall conclusion, it says that based on critical assessment of relevant information, nonylphenol and its ethoxylates are considered to be "toxic" as defined in Section 64 of CEPA 1999 (the Canadian Environmental Protection Act, 1999).

(2) European Union (EU)

The European Commission submitted the final report of the "European Union Risk Assessment Report - 4-Nonylphenol(branched) and nonylphenol" to the European Union in April, 2001⁷⁾.

The report covers the properties, classification, production, uses (including those of nonylphenol ethoxylates), market trends, legislative controls, sources, degradation in the environment, distribution in the environment, environmental concentrations, general toxicity, endocrine disrupting activities, etc. of nonylphenol and its ethoxylates, and makes risk assessments on human health and ecological effects in comparisons with predicted exposure levels and predicted no-effect levels.

In its human risk assessment, the report compares 1.5mg/kg/day- that is - the predicted no-effect level obtained by multiplying the predicted no-observed-adverse-effect level (NOAEL) on reproduction 15 mg/kg/day according to the report of three-generation rat study of U.S.NTP by an assessment factor of 1/10 to 0.6 [g/kg/day that is the predicted exposure level to consumers. It says the comparison leads a margin of safety to 2,500, so that there are no substantial risks to human health. It should be noted that the predicted exposure level to consumers is the sum total of the calculated predicted values of inhalation exposure level (SCIEN model, U.S.EPA) and dermal exposure level (DERMAL model, U.S.EPA) supposing indoor dissemination of mildew-proofing agents, the calculated predicted value of dermal exposure level supposing hair dyeing, and the calculated predicted value of oral exposure level supposing elution from food packing materials.

In its ecological risk assessment, the results show 0.33 [g/L as the predicted no-effect concentration in water (PNEC_{water})(*) by dividing the 10% effect value 72hEC_{10(Biomass)} of 3.3 [g/L for freshwater

green alga (*Scenedesmus subspicatus*) by an assessment factor of 10. Further, as the predicted environmental concentration in surface water ($PEC_{\text{surface water}}$)(*), it obtains the PEC_{regional} of 0.6 [g/L] to see the state of pollution in rivers in the region covering $4 \cdot 10^4 \text{ km}^2$ and the $PEC_{\text{Continental}}$ of 0.066 [g/L] covering a broader $3.56 \cdot 10^6 \text{ km}^2$, according to the calculation results using the EUSES (the European Union System for the Evaluation of Substances) model (fugacity model level III). Then it compares PNECs to and PECs. Noting the $PEC_{\text{regional}}/PNEC$ value is about 1.8, exceeding 1, and the high PEC_{local} values of $\phi 0.6$ to 350 [g/L] obtained to predict the concentrations in the neighborhood of release sources by multiplying concentrations in effluents by dilution rates, it concludes that it is necessary to reduce risks in water environment.

* NB.) PNEC: The concentration in which no effect is predicted.

PEC: Predicted concentration in water.

PEC/PNEC value: If PEC exceeds PNEC (in other words, $PEC/PNEC > 1$), in EU, risk reduction measures are to be required.

11. Overall assessments

Stated below are the results of the risk assessments about the effects of nonylphenol on fish, based on the results of our literature search on endocrine effects activities and the results of toxicity assessment by screening, test, etc., as well as the results of environmental surveys and the reports on environmental behaviors.

(1) Methods of risk assessment

As for the risk assessment about effects on fish, methods of assessment based on exposure levels via feeds and loads/concentrations in fish body are well developed. As for the effects by nonylphenol, however, it is supposed that exposure via feeds is low in proportion. Therefore, it was considered appropriate to use a risk assessment method of obtaining predicted environmental concentration (PEC) and predicted no-effect-concentration (PNEC) to compare, as generally used in Japan and other countries.

(2) Assessment of nonylphenol exposure

(a) Summary of environmental survey results

According to the results of the environmental surveys from FY1998 through FY1999 conducted by the Environment Agency and the Ministry of Construction, the detected concentrations in the water quality surveys ranged from NDs (<0.03 - 0.1) to 21 [g/L]. The mean value of these concentrations was 0.17 [g/L] if calculated assuming NDs are zero, 0.19 [g/L] if NDs are half of detection limit values, and 0.22 [g/L] if NDs are equal to detection limit values. The 75th percentile, 90th percentile and 95th percentile concentrations were 0.10 [g/L], 0.30 [g/L] and 0.59 [g/L], respectively. Referring to the distribution of concentrations of all samples, it was evident that there are two groups of different characters with approximately 95th percentile as a border line.

(b) Environmental behaviors and ecology

The main exposure pathway of nonylphenol to fish is the intake through gills or body surface of fish from water, and exposure through feeds is several percent or lower of the total exposure.

Further, nonylphenol concentration in fish body amounts to tens to hundreds times as high as that in water, but its half life period in fish body is short, for 19-20 hours, as mentioned before. It is supposed, therefore, that most of nonylphenol intake will be excreted outside the body if concentration in water is lowered, and that concentration in fish body will respond well to concentration in water.

Therefore, it is considered to be proper to use concentrations in water in the assessment of

exposure of nonylphenol to fish.

In the surveys conducted in Switzerland and in rivers flowing into Lake Biwa in Japan, no significant differences were observed in concentrations in the bodies of organisms at different trophic stages, giving no evidence that nonylphenol is concentrated in organisms through food chains.

(c) Predicted environmental concentration (PEC)

Environment Canada/Health Canada calculate the predicted environmental concentration (PEC) by multiplying the largest of concentration values in effluents obtained from literature information by a dilution rate. The European Commission calculates the PEC_{local} to predict concentration in the neighborhood of the release, the $PEC_{regional}$ to see the general state of pollution in rivers, and the $PEC_{continental}$ covering wider areas, by model calculations based on the data of annual releases at several sources submitted by industries.

In Japan, data on concentrations in effluents nor annual releases at source is not available, so that it is not possible to make calculations as done by Environment Canada/Health Canada, nor calculate PEC_{local} , etc. as done by the European Commission. Thus, as a concept close to $PEC_{regional}$ calculated by the European Commission, it was decided to estimate an exposure level in the reasonable worst case from the environmental survey results, and adopt it as a representative value. In concrete, it is proposed to classify measuring samples into the general water areas and the areas with high possibility of being affected directly from the release sources, based on the distribution curve of the survey results, and to adopt provisionally the 95th percentile concentration of 0.59 [g/L, that is the highest value estimated in the general water areas, as the predicted environmental concentration (PEC).

In the future, it must be considered to calculate the predicted environmental concentration (PEC) using available data on releases and concentrations in effluents from the sources in Japan and the newly developed water environment behavior model.

(3) Hazard assessment of endocrine disrupting effects of nonylphenol on fish

Within the literature information of 1972-2000 obtained through TOXLINE, etc., in-water nonylphenol concentrations suspected to have endocrine disrupting effects on fish in the reported test results, whose reliability was confirmed, were 1.6 [g/L where abnormality in testis tissue of fathead minnow was observed in electron microscopic examination, 10 [g/L where vitellogenin mRNA was induced in liver of juvenile rainbow trout, 20.3 [g/L where vitellogenin was produced in plasma of mature male rainbow trout (the threshold value was estimated at 10 [g/L in the report), etc.

Within the *in-vitro* test results, nonylphenol showed the relative strength of binding to estrogen receptor at 1/10, as compared with E_2 , in medaka receptor binding assay, and at 1/200 in mummichog receptor binding assay, and the transcription activating power at a several-hundredth, as compared with E_2 , in medaka reporter gene assay. Although, it was reported that nonylphenol's binding affinity was in the range of 1/2,000 to 1/3,000, as compared with E_2 , in the test of binding to estrogenic receptor using Atlantic croaker, but the test concerned examined responses not in the receptor alone, but also in the cell from which its cytosol was extracted along with its surrounding cells. So the reported data is deemed lacking in reliability, as compared with MoE's test series examining genuine binding to receptor.

Within the screening results, in male medaka vitellogenin assay, significant production of vitellogenin was observed at concentration of 22.5 [g/L in water (NB.: not observed at ϕ 12.8 [g/L), and in medaka partial life cycle test, feminization of males in secondary sexual character at concentration of 23.5 [g/L in water, and appearance of testis-ovas and production of vitellogenin at concentration of 11.6 [g/L in water were observed significantly (NB.: not observed at ϕ 6.08 [g/L).

Further, in medaka full life cycle test, abnormality in sex differentiation of males, decrease in fertilization rate, etc. were observed at concentration of 17.7 [g/L in water, and testis-ovas not observed in the first generation were observed in the second generation at 8.2 [g/L (NB.: not observed at 4.2 [g/L).

As for nonylphenol, it has been reported in the past that vitellogenin was induced at low concentrations, indicating suspected endocrine disrupting activity to fish. However, there are many unknown points concerning vitellogenin, including the fact that while it is deemed peculiar to female, it was also observed in male fish not exposed to nonylphenol. As a result, vitellogenin was only used as a biomarker in screening techniques, and could not become an index to judge the existence or extent of endocrine disrupting activities. Under these circumstances, it may be safely said that this is the first evidence in the world to show, with regard to the suspected endocrine disrupting effects of nonylphenol, that morphogenetic abnormality such as testis-ova was observed at low concentrations in the test using medaka, sex ratio of which hardly changes despite environmental change. In supporting this, it was proved in *in-vitro* tests that nonylphenol has strong binding affinity to estrogen receptor and strong estrogenic effects on fish, though varied widely by fish species. As mentioned above, it was strongly supposed that nonylphenol has strong endocrine disrupting effects on fish.

(a) Setting of No-Observed-Effect Concentration (NOEC)

The results of the literature search and screening tests can be summarized below:

- (i) Abnormality in testes as observed in fathead minnow is a change observed in electron microscopic examination, and not a statistically significant change;
- (ii) In medaka full life cycle tests, no significant difference in appearance of testis-ova was observed at concentration of 8.2 $\mu\text{g/L}$ in water; and
- (iii) it is hard to believe that induction of vitellogenin mRNA in rainbow trout observed at 10 $\mu\text{g/L}$ has significant direct effect on the organism, and the concentration is the lowest one in the test, from which NOEC cannot be obtained.

It was thought that these results should be used only for reference. On the other hand, considering that in the medaka full life cycle test, abnormality in sex differentiation and decrease in fertilization rate were observed at concentration of 17.7 $\mu\text{g/L}$, though not to the extent of affecting conservation of species, and that, in medaka partial life cycle test, testis-ovas and vitellogenin production were observed significantly at concentration of 11.6 $\mu\text{g/L}$ in water, at which nonylphenol is believed to have significant effect on sex differentiation, it is considered appropriate to adopt 11.6 $\mu\text{g/L}$ as the Lowest-Observed-Effect Concentration (LOEC). This value is close to the concentration at which vitellogenin mRNA was induced in rainbow trout.

In this case, NOEC will be 6.08 $\mu\text{g/L}$. This value is close to the NOEC of 7.4 $\mu\text{g/L}$ presented by the European Commission as chronic toxicity to fish concerning to survival observed in a test using fathead minnow as test organism ⁷⁾, and to 6 $\mu\text{g/L}$ presented by Environment Canada/Health Canada as NOEC for fish ¹⁷⁾.

(b) Setting of Predicted No-Effect Concentration (PNEC)

As for PNEC, it is conceivable, in a more conservative approach in view of the limited number of species as test organism, to adopt an assessment factor in the range of 100 to 1,000 as used by other OECD countries. Considering that the effects concerned are not classified as classical acute or chronic toxicity, and they are not lethal effects, it is proposed to adopt 0.608 $\mu\text{g/L}$ as PNEC by dividing NOEC by an assessment factor of 10. This value covers the concentration (1.6 $\mu\text{g/L}$) causing reproductive abnormalities observed in fathead minnow, too.

Further, if the endocrine disrupting effects observed at NOEC suspected of having can be considered to be part of chronic toxicity in a broad sense, the assessment factor of 10 adopted by MoE is also deemed proper. Since in those case where effective concentrations for algae and crustacea have already been obtained as chronic toxicity. Other OECD countries have adopted 10 as the assessment factor.

As for human health effects, on which MoE is now conducting various tests, in the *in-vitro* tests using human cells, nonylphenol's binding to estrogenic receptor is very weak, and in the tests using rodents as obtained through literature search, no reaction at very low concentrations has

not been reported (see Appendix 3), it should be noted that the results obtained in fish would not necessarily apply to human health.

(4) Other effects of nonylphenol to fish

Among the concentrations, lower than NOEC value of 6.08 [g/L, at which any effect as general toxicity was reported, (i) as acute toxicity, there is a 10%-effective concentration (EC₁₀) of 3.3 [g/L for reproduction observed in a test using freshwater alga (*Scenedesmus subspicatus*) as test organism, but in view of the degree of ecological effect, it is controversial internationally whether or not to adopt this value as PNEC, and (ii) as chronic toxicity, there is an NOEC of 3.9 [g/L for growth observed in a test using mysids (marine invertebrate) as test organism, but nonylphenol is detected mainly in rivers, so that freshwater organism should be used for an index of nonylphenol toxicity to be used in this risk assessment. From these consideration, it is decided not to adopt any of said concentrations as an index of toxicity for this present risk assessment. In any case, these value levels are covered by the PNEC adopted by MoE.

(5) Others (environmental fate and metabolism, internal fate and metabolism, etc.)

Because of few scientific data on nonylphenol behaviors, etc. in the fish body, it seems difficult to make risk assessment, at present, based on nonylphenol concentrations in the body.

Also, in order to make risk assessment in accordance with the actual state of affairs in Japan, at present, it is deemed lacking in data on concentrations in water in the neighborhood of individual release sources at high concentrations.

According to Environment Canada/Health Canada, nonylphenol and nonylphenol ethoxylates are present in effluents from their release sources at high concentrations, but they say constant monitoring of effluents is necessary because this is a possibility of higher concentrations and big fluctuations¹⁷⁾.

(6) Risk assessment of nonylphenol's effects on fish

Based on toxicity assessment, the Predicted No-Effect Concentration (PNEC) of nonylphenol is 0.608 [g/L obtained by dividing the No-Observed-Effect Concentration (NOEC) of 6.08 [g/L by an assessment factor of 10, and this value is close to the Predicted Environmental Concentration (PEC) of 0.59 [g/L adopted in the exposure assessment. Concentrations of nonylphenol in environmental water in Japan observed in the environmental surveys ranged from NDs (<0.03-0.1) to 21 [g/L, and the concentrations at 71 samples (4.5%) of total 1,574 samples exceeded the PNEC value.

It is assessed, therefore, that nonylphenol observed in ambient water in Japan could have ecologically be affected through endocrine disrupting effects on fish.

As for the PEC, a representative value of the environmental surveys is provisionally adopted in a concept close to PEC_{regional} used by the European Commission to see the general state of pollution in rivers. It should be noted, however, that the said environmental surveys have been conducted in general areas, and their results do not represent the state of pollution in the water area surrounding sources, and furthermore that other substances having similar estrogenic effects, such as natural and synthesized estrogens, have already been present in the environment with possible complex effects, as well.

12. Measures toward risk reduction

Based on the summarized risk assessment results on the effects of nonylphenol on fish, as mentioned above, it is considered that the following efforts should be made:

(1) Further improvement of accuracy in risk assessments

The present risk assessments have, as the first case, assessed toxicity on the chemicals strongly suspected of having endocrine disrupting effects both *in vitro* and *in vivo* tests. These hazard assessments

have been conducted based on the highly reliable data, but the screening and test methods to assess endocrine disrupting effects are not established yet, and the efforts to develop such methods are now under way on an OECD basis. Under these circumstances, MoE's present assessments have been provisionally carried out using existing toxicity assessment methods, therefore further accumulation of scientific data is required to increase accuracy as well.

As for the exposure assessment, the measuring sites covered in the past environmental surveys by MoE were selected, centering around representative rivers, etc. in each prefecture. In the future, however, for the purpose of making comprehensive exposure assessments, and with a view to calculating PEC_{local} to predict concentrations in the neighborhood of release sources, it is important to grasp release sources using the PRTR system, etc. and carry out detailed surveys in the surrounding areas in cooperation with local municipalities and related ministries/agencies to confirm the state of environmental pollution in more detail. By doing so, it is necessary to continuously improve exposure assessments to take effective measures as early as possible.

(2) Tackling toward risk reduction

Considering the results of our present risk assessments, it is deemed necessary to take measures to reduce risk in view of ecological conservation.

As for alkylphenols, especially nonylphenol, various efforts are already being made nationally and internationally to prevent environmental prevention, based on the existing data mainly on their general toxicity having ecological effects and their environmental concentrations. Especially in foreign countries taking lead in this field, various legislative regulations and industry measures are being widely executed toward reduction in use of alkylphenol ethoxylates (including nonylphenol ethoxylates) (see Appendix 2). In Japan, too, related industries are taking such voluntary measures as to disuse alkylphenol ethoxylates in cleansers for household use and to promote use of alternative substances in detergents for business and industrial uses.

In this present risk assessment, it is assessed that nonylphenol could have effects on ecology, centering around fish. Since regulations on chemical substances in Japan are mainly aiming at human health protection rather than ecological conservation, it is important to examine how to tackle the problems about nonylphenol suspected of having ecological effects in reviewing Japanese overall measures on chemical substances.

Including this point, consideration should be given to the following matters:

- (a) It is necessary to examine promptly, among the parties concerned, the measures to reduce concentrations in water environment to the predicted no-effect concentration (PNEC) or lower. In this respect, it is expected for the related industries to take autonomous measures to promote use of substitute substances. In additions by making use of the PRTR system, etc, it is also expected for them to further promote voluntary management for risk reduction.
- (b) In the development and use of substitutes, careful selections are required because selected substitutes could lead to serious effects on human health and the environment even though their toxicity is not expected at present. It is necessary to promote the use of high-degradability, low-toxicity and ecology-friendly substitutes, and to speed up such efforts through the tripartite collaboration of industry, academia and government. In view of MoE's literature search and various test results so far (see Appendix 1), it is deemed improper to use 4-*t*-octylphenol as a substitute because it is suspected to have toxicity and endocrine disrupting effects similar to nonylphenol. Any use of other branched alkylphenols also requires careful examination in the light of *in vitro* test results.

On the other hand, in the light of its environmental behaviors and internal fate observed so far, nonylphenol has some degradability in the environment and is expected to be excreted outside the body in a relatively short period, so that the need to take measures to reduce or remove the existing nonylphenol in the environment is deemed low.

- (c) In order to control chemicals having serious effects on the environment, rather than human health, it is necessary for the government to have discussions on (i) setting up goals for water quality with a view to ecological conservation with relevant measures to be taken to achieve them, and (ii) how the evaluations and regulations on chemicals should be with a view to ecological conservation.

(Appendix 1) Other alkylphenols

1. 4-*t*-Octylphenol

(1) Summary of the environmental survey results

Concentrations of 4-*t*-octylphenol in the water, bottom sediment, soil, aquatic organism and wildlife at a total of 2,331 samples all over Japan were measured in the Nationwide Urgent Endocrine Disrupters Survey (1998) and the Nationwide 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. As a result, in the water quality survey, 4-*t*-octylphenol was detected at 328 samples of total 1,574 samples in two years (detection rate: 21 %), and its concentration range was NDs (<0.01-0.1) - 13 [g/L. The arithmetic mean concentration was 0.02 [g/L (calculated assuming NDs = 0), median and 75th percentile concentrations were ND, 90th percentile 0.03 [g/L, and 95th percentile 0.06 [g/L. The arithmetic mean concentration was 0.04 [g/L and 0.05 [g/L if calculated assuming that NDs are half of the detection limit values and equal to them, respectively.

In the bottom sediment survey, 4-*t*-octylphenol was detected at 60 samples of total 294 samples in two years (detection ratio: 20 %), and its concentration range was NDs (<1-10.5) - 170 [g/kg. The arithmetic mean concentration was 3.5 [g/kg (calculated assuming ND = 0), median and 75th percentile concentrations were ND, 90th percentile 9.0 [g/kg, and 95th percentile 16 [g/kg. But the arithmetic mean concentration was 4.9 [g/kg and 6.4 [g/kg if calculated assuming that NDs are half of the detection limit values and equal to them, respectively.

In the aquatic organism survey, 4-*t*-octylphenol was detected at 16 samples of total 141 samples (detection rate: 11 %). Its concentration range was NDs (<1.5) - 30 [g/kg. The arithmetic mean concentration was 0.9 [g/kg (calculated assuming ND = 0), median and 75th percentile concentrations were ND, 90th percentile 1.8 [g/kg, and 95th percentile 4.8 [g/kg. But the arithmetic mean concentration was 1.6 [g/kg and 2.3 [g/kg if NDs are half of the detection limit values and equal to them, respectively.

(2) Literature search and reliability assessment results on ecological effects suspected of endocrine disrupting effects

Of the literature information of 1972-2000 obtained through TOXLINE, etc., the following reports which showed suspected endocrine disrupting effects on fish and observed such activities, and their reliability was acknowledged in the reliability assessment conducted by MoE.

- Jobling et al.⁵⁸⁾ studied effects on mature male rainbow trout exposed to 4-*t*-octylphenol at concentrations of 0.3, 0.6, 1.6, 4.8, 14.6 or 43.9 [g/L (measured values) for 3 weeks. As a result, in the group exposed to 4.8 [g/L or higher, induction of vitellogenin was observed in plasma.
- Gronen et al.⁶⁸⁾ studied effects on male medaka exposed to 4-*t*-octylphenol at concentrations of 20, 41, 74 or 230 [g/L (measured values) for 21 days. As a result, in the group exposed to 20 [g/L or higher, vitellogenin was synthesized in plasma and abnormalities were observed in reproductive behavior.
- Pedersen et al.⁶⁰⁾ studied effects on juvenile rainbow trout exposed to 4-*t*-octylphenol at concentration of 41 [g/L (measured value) for 9 days. As a result, increase in vitellogenin concentration was observed in plasma.
- Bayley et al.⁶⁹⁾ studied effects on male guppies exposed to 4-*t*-octylphenol at concentration of 150 [g/L for 4 weeks. As a result, effects on sexual behavior were observed.

(3) Examination of toxicity assessment on endocrine disrupting effects

Of the literature information of 1972-2000 obtained through TOXLINE, etc., the concentration levels of 4-*t*-octylphenol in water suspected to have endocrine disrupting effects on fish in the reported test results, whose reliability was confirmed, were 4.8 [g/L where vitellogenin was synthesized in plasma of mature male rainbow trout⁵⁸⁾, 20 [g/L where vitellogenin was synthesized in serum of mature male medaka and abnormalities were observed in reproductive behavior⁶⁸⁾, etc.

Of the *in-vitro* test results this time, 4-*t*-octylphenol showed the relative strength of binding to estrogen receptor at 1/5, as compared with E₂, in medaka receptor binding assay, and at about 1/150 in mummichog receptor binding assay, and showed the transcription activating power at several-hundred times higher concentration than that of E₂ in medaka reporter gene assay.

Of the screening results described in this report, in male medaka vitellogenin assay, significant production of vitellogenin was observed at concentration of 64.1 [g/L in water (NB.: not observed at ϕ 27.8 [g/L), and in medaka partial life cycle test, feminization of males in secondary sexual character at concentration of 48.1 [g/L in water, and appearance of testis-ovas and production of vitellogenin at concentration of 11.4 [g/L in water were observed significantly (NB.: not observed at ϕ 6.94 [g/L).

In the future, medaka full life cycle tests will be carried out to assess the effects of 4-*t*-octylphenol. Putting the results of these tests together, effects are expected to be observed at concentrations of 4-*t*-octylphenol in water similar to those of nonylphenol, including differences by fish species.

As mentioned in the section of nonylphenol, it should be noted that the test results in fish cannot be extrapolated to human health as it is.

2. Other alkylphenols

(1) Summary of the environmental survey results

According to the environmental surveys conducted by MoE in FY 1998 to FY 1999, concentrations of 4-*t*-pentylphenol in ambient water ranged from NDs (<0.01) to 0.02 [g/L, and it was only detected in 3 of total 916 samples (detection ratio: 0.3 %).

(2) Literature search and reliability assessment results on ecological effects suspected of endocrine disrupting effects

Among other alkylphenols, information on 4-*t*-pentylphenol was obtained. Concentrations of 4-*t*-pentylphenol in water, at which reliability of the results suspected of having endocrine disrupting effects on fish was confirmed, were 32 [g/L at which gonadosomatic index of mature male carp decreased significantly⁷⁰⁾, and 100 [g/L at which oviduct was formed in male carp⁷⁰⁾, etc.

(3) Examination of toxicity assessment on endocrine disrupting effects

From the literature information of 1972-2000 obtained through TOXLINE, etc., information on 4-*t*-pentylphenol was obtained among other alkylphenols. Concentrations of 4-*t*-pentylphenol in water, at which reliability of the results suspected of having endocrine disrupting effects on fish was confirmed, were 32 [g/L at which gonadosomatic index of mature male carp decreased significantly⁷⁰⁾, and 100 [g/L at which oviduct was formed in male carp⁷¹⁾, etc.

Of the *in-vitro* test results this time, 4-*t*-pentylphenol relative binding strength to estrogen receptor was 1/100, and 4-*t*-butylphenol 1/500, as compared with E₂, in medaka receptor binding assay.

Though not included in the screening and testing this time, full life cycle test using 4-*t*-pentylphenol has been conducted earlier, since this chemical is an OECD reference material. According to said test, among males, abnormality in sex differentiation, decrease in fertilization, etc. were observed at in-water concentration of 224 [g/L (not observed at 100 [g/L), and testis-ovas were observed in the second generation at concentration of 51.1 [g/L, though not observed in the first generation. From chemical structural formula, accumulation of 4-*t*-pentylphenol in fish body is expected to be lower, compared

with nonylphenol. Further, relative binding strength *in vitro* test was 1/100, as compared with E₂. Considering these points, 4-*t*-pentylphenol has estrogenic effects on fish, but its effective concentration was at least several tens μg/L or higher, and its effect level is supposed to be weak, compared with nonylphenol and 4-*t*-octylphenol.

(Appendix 2) Regulations, etc. in Japan and other countries

(1) Regulations in Japan

Nonylphenol is subject to the Law Concerning Reporting, etc., of Release of Specific Chemical Substances to the Environment and Promotion of the Improvement of Their Management (so-called the Pollutant Release and Transfer Register (PRTR) Law), as well as to the Law Relating to the Prevention of Marine Pollution and Maritime Disaster. It is also an "item requiring environmental survey" in the list of priority substances subject to the measures to be taken against effects on water environment.

Further, nonylphenol is designated as a substance subject to control, in view of its corrosiveness, in the Fire Prevention Law, the Ship Safety Law, the Aviation Law and the Port Regulation Law.

It is not subject to the Law Concerning the Examination and Regulation of Manufacture, etc., of Chemical Substances, the Industrial Safety Health Law, the Water Pollution Prevention Law and the Air Pollution Prevention Law.

Appendix Table 1 Japanese regulations on nonylphenol

Name of law	Type of regulation
Law Concerning Reporting, etc., of Release of Specific Chemical Substances to the Environment and Promotion of the Improvement of Their Management	Class I Specified Chemical Substances (substances subject to PRTR System and MSDS System)
Law Relating to the Prevention of Marine Pollution and Maritime Disaster	Group A substances (harmful substances in view of marine environment)
Fire Prevention Law, Ship Safety Law (Regulations for Carriage and Storage of Dangerous Goods in Ships), Aviation Law	Corrosive substances

(2) Regulations in other countries

As for nonylphenol the European Union (EU) is examining the necessity of regulation under the Council Directive 67/548/EEC on the approximation of the laws, ordinances and administrative rules relating to the classification, packaging and labeling of dangerous substances. The European Union Risk Assessment Report on nonylphenol prepared by the United Kingdom on behalf of the EU was reportedly agreed at the EU level, but not published yet ⁷⁾.

Switzerland banned the use of octylphenol ethoxylates and nonylphenol ethoxylates in cleansers, detergents and cleaning auxiliaries under the law on environmentally harmful substances in 1987 ⁷²⁾.

Denmark set limit values on nonylphenol and nonylphenol ethoxylates contained in the wastes for agricultural use (fertilizer and sludge). The program to disuse nonylphenol ethoxylates in agricultural chemicals is in operation ⁷²⁾.

In Norway, the government adopted in October, 1996, the decision to phase out all alkylphenols by the end of 2000 at the latest, and has made clear its policy not to permit pesticides containing alkylphenol or alkylphenol ethoxylates. Further, the government has prohibited the use of these substances in food packing ⁷²⁾.

In Canada, the risk assessment report (draft) on nonylphenol and nonylphenol ethoxylates was published in March, 2000 ¹⁷⁾. The draft report proposes to designate these substances as harmful substances under Canadian Environmental Protection Law, in view of the environment and biodiversity. The report was made available for public comments in April and May, 2000, and is now under revision work.

Looking at aquatic organism criteria, U.S. Environmental Protection Agency is examining the establishment of aquatic organism criteria for nonylphenol, but nonylphenol is not subject to the Cana-

dian aquatic organism guideline or the Australian aquatic organism criteria.

(3) Voluntary measures taken by industries

(a) Tackling in Japan

In our country, Japan Soap and Detergent Association (JSDA), Japan Soap and Detergent Industry Association, and Japan Surfactant Industry Association are making autonomous efforts toward disuse of alkylphenol ethoxylates ¹¹⁾.

Appendix Table 2 Autonomous tackling to reduce the use of alkylphenol ethoxylates in Japan

Japan Soap and Detergent Association (JSDA)	<p>In April 1998, made the following announcement: ↓At its board of directors meeting in September 1996, it was decided that its member companies should not use alkylphenol ethoxylates in detergents for household use. As of the announcement, its member companies did not use alkylphenol ethoxylate in detergents for household use.</p>
Japan Soap and Detergent Industry Association	<p>At its board of directors meeting in July 1999, it was confirmed that its member companies did not use alkylphenol ethoxylates in detergents for household use and would not use them in the future, either.</p>
Japan Surfactant Industry Association	<p>At its board of directors meeting in March 1999, it was decided that its member companies should take the following steps: ↓Check on their customers' use of alkylphenol ethoxylates, and persuade any customer using alkylphenol ethoxylates in detergents for household use to use substitutes. ↓Promote polyoxyethylene alkylether, etc., as substitutes, since substances used in detergents, etc., for business and industrial uses are apt to be released directly into the environment. ↓Promote substitution in other areas, too, if possible.</p>

(b) Measures taken in Europe

In Europe, industries are taking voluntary measures to reduce the use of nonylphenol and nonylphenol ethoxylates. The measures taken in European countries are shown below in Appendix Table 3 ⁷²⁾.

Appendix Table 3 Voluntary measures to reduce the use of nonylphenol and nonylphenol ethoxylates in Europe

OSPARCOM	Recommended the following in 1992: Goal 1: Phase out nonylphenol ethoxylates for cleaning use in households by 1995. Goal 2: Phase out nonylphenol ethoxylates as cleaning material for industrial use by 2000.
Denmark	The government declared that alkylphenol ethoxylates contained in pesticides should be phased out by 2000, in view of their estrogenic activities.
Germany	Alkylphenols in detergents for household use were abolished by 1995.
Norway	An agreement is made between the government and industries that detergents for household use should be abolished by 1995, and those for industrial use by 2000.
Sweden	Nonylphenol ethoxylates in detergents for household use were disused in 1973, and those in cleaning products for household use had been disused by 1992.
Belgium	The government and manufacturers agreed to abolish nonylphenol ethoxylates for household use by 1995 (NB.: the achievement of this goal was confirmed), and those for industrial use by 2000.
Netherlands	Nonylphenol ethoxylates are not used as cleaning chemical in households since 1998. Alkylphenols are not used as cleaning material since 1995.
United Kingdom	The government and cleaning material manufacturers agreed to disuse nonylphenol ethoxylates in household products, and abolished them completely in effect. Also, an agreement is made between the U.K. environmental agency and the U.K. wool textile industry that alkylphenols in wool polishing agents used in the northwestern part of U.K. should be substituted by other substances.

(4) The state of substitution

It is said that most of alkylphenols can be substituted by nonionic surfactants, such as straight alcohol ethoxylates (polyoxyethylene alkyl ether, etc.), fatty acid and its derivatives, fatty amine, or unsaturated hydrocarbon.

Appendix Table 4 shows the state of substitution for alkylphenols in Japan (partially including estimate).

Appendix Table 4 The state of substitution for alkylphenols in Japan (partially including estimate)

Rubber, plastics	Polyoxyethylene alkyl ether, sodium alkyldiphenyl ether disulfonate, etc.
Textile	Polyoxyethylene alkyl ether, (-olefin sulfonate, vegetable soap, etc.
Machinery, metal, information equipment and transport equipment	Polyoxyethylene polyoxypropylene block polymer, polyoxyethylene fatty acid ester, polyoxyethylene alkyl ether, etc.
Dye, pigment, paint, ink	Polyoxyethylene alkyl ether
Agricultural chemical, disinfectant, fertilizer, feeds	Sodium dioctyl sulfosuccinate
Civil engineering, building, ceramics	N-alkyl trimethylene diamine
Pharmaceutical, cosmetics & toiletries	Glycerol monostearate, sorbitan fatty acid ester, polyoxyethylene sorbitan fatty acid ester
Leather goods	Polyoxyethylene alkyl ether
(For reference) Cleaning, paper & pulp, petroleum, mining, tar, fuels	Continued use

(Appendix 3) On the reports on effects of nonylphenol to mammals

1. Fate in the body

The report obtained through JICST is stated below. Reliability assessment is not made by MoE.

- Knaak et al.⁷⁵⁾ studied metabolism of nonylphenol (kind of isomer not known) orally or intraperitoneally administered to male rats at 1mg/150g body weight. As a result, in either administration method, about 90-95% of nonylphenol was excreted as feces and urine (about 70-75% into feces, and about 20% into urine) within 7 days. The metabolite in urine was conjugated with glucuronic acid.

2. General toxicity

(1) Acute toxicity

The reports on acute toxicity as reported by the European Commission or Environment Canada/Health Canada are as follows. Reliability assessment is not made by MoE.

According to the reports by the European Commission or Environment Canada/Health Canada, values of acute toxicity to human health were not obtained.

- The European Committee reported oral acute toxicity tests by Berol Kemi⁷⁶⁾, Huls⁷⁷⁾ and ICI⁷⁸⁾ using rat as test organism⁷⁾. As a result, median lethal dose (LD₅₀) ranged from 1,200 to 2,400 mg/kg. Also, oral acute toxicity test by Gaworski et al.⁷⁹⁾ using mouse as test organism was reported. As a result, LD₅₀ was 307 mg/kg.
- Further, dermal acute toxicity test by Smyth et al.⁸⁰⁾ using male rabbit as test organism. As a result, LD₅₀ was 2,031 mg/kg.
- Environment Canada/Health Canada reported LD₅₀ for rats at 580-1,620 mg/kg¹⁷⁾.

(2) Chronic toxicity (repeated administration toxicity)

The reports of chronic toxicity as reported by the European Commission or Environment Canada/Health Canada are stated below. Reliability test is not made by MoE.

- The European Commission reported the test by Huls⁸¹⁾ on the effects of nonylphenol on male and female rats administered mixed feeds containing nonylphenol at 25, 100 or 400 mg/kg/day for 28 days⁷⁾. As a result, only in males in the 25 or 100 mg/kg/day administered group, slight increase in kidney weight, adrenal weight and liver weight, and slight traces of glass drop formation were observed. In the 400 mg/kg/day group, low values of body weight increase were observed, and in males alone, slight increase in urea value and cholesterol value, slight decrease in glucose value, increase in relative liver weight and relative testis weight to body weight, accumulation of glass drops in kidney renal tubule, and slight vacuolation of liver cells surrounding portal vein were observed. The No-Observed-Adverse-Effect Level (NOAEL) was estimated at 100 mg/kg/day.
- The European Commission also reported the test by Cunny et al.⁸²⁾ on the effects on male and female SD Crl:CD BR rats administered mixed feeds with the feed containing 4-nonylphenol (branched, purity: 95.6%) at 200, 650 or 2,000 ppm for 90 days⁷⁾. Intake were calculated at 15, 50 or 140 mg/kg/day, respectively. As a result, in the 140 mg/kg/day intake group, low values of increase in body weight were observed; increase in kidney weight and relative kidney weight to body weight as well as decrease in glass drops in kidney renal tubule were observed in males alone, and slight decrease in ovary weight in females. The NOAEL was estimated at 50 mg/kg/day.
- U.S.NTP⁸³⁾ tested three generations of SD rats by administering mixed feeds using 4-nonylphenol (branched) as test substance. Average intake of 4-nonylphenol (branched) was calculated at 15 mg/kg/day (males: 12-18 mg/kg/day, females not in lactation period: 16-21 mg/kg/day, females in lactation period: 27-30 mg/kg/day), 50 mg/kg/day (males and females not in breeding period: 43-64 mg/kg/day, females in lactation period: 93-98 mg/kg/day), or 160 mg/kg/day (males and fe-

males not in breeding period: 131-199 mg/kg/day, females in lactation period: 274-332 mg/kg/day). As a result, in the males of all generations and F₃ females in the intake groups of 15 mg/kg/day or higher, increase in denaturation or dilatation of kidney renal tubule was observed. In F₁ females, F₂ males and F₃ females in the 50 mg/kg/day intake group, low values of increase in body weight were observed. In F₀ males and F₂ males in the intake group of 50 mg/kg/day or higher, increase in relative kidney weight to body weight was observed. In the 160 mg/kg/day intake group, low values of body weight increase were observed in all generations, increase in relative kidney weight to body weight was observed in F₁ males and females, and increase in denaturation or dilatation of kidney renal tubule was observed in F₁ females, F₂ females and F₃ females. The LOAEL was estimated at 15 mg/kg/day (12-18 mg/kg/day).

- Environment Canada/Health Canada also reported the study by Richards⁸⁴⁾ in rats administered nonylphenol in mixed feeds for 28 days¹⁷⁾. As a result, Increase in relative liver weight to body weight was observed in the 25 mg/kg/day administered group.

(3) Reproductive toxicity

Stated below are the reports on reproductive toxicity to mammals as obtained from the literature information of 1966-2000 through MEDLINE, etc. and as reported by the European Commission or Environment Canada/Health Canada. Reliability assessment is not made by MoE. In the reported tests, assessment items (end points) suspected as endocrine disrupting effects, too, were adopted, and responses were observed. Due to the fact that their set doses were extremely high, etc., however, it is hard to deem them as tests to see the existence or extent of endocrine disrupting effects, thus, it was considered appropriate to treat them as tests for reproductive toxicity.

- In the test by U.S.NTP⁸³⁾ using three generations of SD rats, as stated above, reproductive toxicity was studied, too. As a result, in the intake group of 50 mg/kg/day or higher, earlier vaginal opening was observed in females of all generations, increase in relative uterus weight to body weight in F₁ female, decrease in ovary weight in F₂ females, and decrease in spermatic concentrations in the testis upper part in F₂ males. In the intake group of 160 mg/kg/day, extension of estrous cycle in F₁ and F₂ females, decrease in ovary weight in females of all generations, and decrease in the number of sperm in testis in F₂ males were observed. The NOAEL was estimated at 15 mg/kg/day.
- Odum et al. studied the effects⁸⁵⁾ on the ovary-removed female Noble rats orally administered *p*-nonylphenol (isomer mixture, branched) at 45, 75, 150 or 225 mg/kg/day for 3 days, or at 53 or 150 mg/kg/day for 11 days, and the effects⁸⁶⁾ on the ovary-removed female Alpk rats orally administered *p*-nonylphenol (isomer mixture, branched) at 100 mg/kg/day for 11 days. As a result, an increase in uterus weight depending on dose was observed in the group of female Noble rats administered 75 mg/kg/day or higher for 3 days, and increase in uterus weight was observed in the group of female Noble rats administered at 150 mg/kg/day for 11 days. Increase in uterus weight was observed in female Alpk rats, too.
- Odum et al.⁸⁶⁾ studied the effects on the female Alpk rats orally administered *p*-nonylphenol (isomer mixture, branched) at 37.5, 75, 150, 225 or 250 mg/kg/day for 3 days, and the effects on the female SD rats orally administered *p*-nonylphenol (isomer mixture, branched) at 250 mg/kg/day for 3 days. As a result, in the group of female Alpk rats administered 75 mg/kg/day or higher, an increase in uterine weight depending on dose was observed. Increase in uterus weight was observed in female Alpk rats, too.
- de Jager et al.⁸⁷⁾ studied the effects on the SD rats orally administered *p*-nonylphenol at 100, 250 or 400 mg/kg/day for 10 weeks. As a result, decrease in diameter of sperm tubule was observed in the group administered 100 mg/kg/day or higher, decrease in testis upper-part weight and relative testis upper-part weight to body weight in the group administered 250 mg/kg/day or higher, and decrease in testis weight, relative testis weight to body weight, and number of sperm in the group administered 400 mg/kg/day or higher. The LOAEL was estimated at 100 mg/kg/day.
- Odum et al.⁸⁸⁾ studied the effects on female Alpk and AP rats orally administered *p*-nonylphenol

(isomer mixture, branched) at 47.5, 95, 190 or 285 mg/kg/day or *p-n*-nonylphenol (isomer mixture, straight) at 285 mg/kg/day for 3 days. As a result, increase in uteris weight was observed in the group administered *p*-nonylphenol at 190 mg/kg/day or higher. In the group administered *p-n*-nonylphenol (isomer mixture, straight), increase in uteris weight was not observed.

- Cunny et al.⁸²⁾ studied the effects on female and male SD CrI:CD BR rats administered mixed feeds with the feed containing 4-nonylphenol (branched, purity: 95.6%) at 200, 650 or 2,000 ppm for 90 days. Intake was calculated at 15, 50 or 140 mg/kg/day. As a result, no effects on estrous cycle was observed in females. In males and females, change in weight of reproductive organs and morphologic change were not observed.
- Coldham et al.⁸⁹⁾ studied the effects on the female CFLP mice hypodermically administered 4-nonylphenol (technical grade) at 0.5, 1, 5 or 20 mg for 3 days. As a result, increase in uteris weight was observed in the group administered at 5 mg or higher.
- Shelby et al.⁹⁰⁾ studied the effects on the female CrL:CD-1(ICR) mice hypodermically administered *p*-nonylphenol (isomer mixture, branched) at 0.001, 0.01, 0.1, 1, 10, 100 or 1,000 mg/kg/day for 3 days. As a result, increase in uteris weight was observed in the group administered at 10 mg/kg/day or higher.
- Odum et al. studied the effects⁸⁵⁾ on the ovary-removed female Noble rats intraperitoneally administered *p*-nonylphenol (isomer mixture, branched) at 0.073 or 53.2 mg/kg/day for 11 days, the effects on the ovary-removed female Alpk rats intraperitoneally administered *p*-nonylphenol (isomer mixture, branched) at 0.037 or 27.2 mg/kg/day for 11 days, and the effects⁸⁶⁾ on the female Alpk rats intraperitoneally administered *p*-nonylphenol (isomer mixture, branched) at 0.052 or 37.4 mg/kg/day for 11 days. As a result, effects on increase in uteris weight or mammary gland multiplication/differentiation were not observed.
- Lee et al.⁹¹⁾ studied the effects on male SD rats in the 1st day of birth intraperitoneally administered nonylphenol (kind of isomer unknown) at 0.08, 0.8, or 8 mg/kg/day for 14 days. As a result, in the group administered at 0.8 mg/kg/day or higher, decrease in relative weight of reproductive organs (testis, testis upper-part, spermatic sac, and prostate) to body weight was observed in the 31st day from the start of administration.
- Milligan et al.⁹²⁾ studied the effects on the ovary-removed Swiss Albino mice hypodermically administered 4-nonylphenol (kind of isomer unknown) at 10^{-6} , 10^{-5} or 10^{-4} M. As a result, rise in uteris blood permeability was observed at administration group of 10^{-5} or higher.
- Nagao et al.⁹³⁾ made a two-generation study on Crj:CD(SD)IGS rats orally administered nonylphenol (isomer mixture, purity: 99.0% or higher) at 2, 10 or 50 mg/kg/day. As a result, in F₀ males in the group administered 50 mg/kg/day, increase in kidney weight and relative weight of liver, kidney, thyroid, hypophysis and lung to body weight, increase in thyroid stimulating hormone value in serum, decrease in thymus weight and relative weight of thymus to body weight, and histological change in liver and kidney were observed; and in F₀ females in the same group, decrease in ovary weight and relative weight of ovary to body weight were observed. Effects on spermatic properties, sexual cycles and ovary tissues were not observed. In F₁ males in the group administered 50 mg/kg/day, increase in follicle stimulating hormone values in serum (22nd day after birth), decrease in thyroid stimulating hormone T₃ value in serum (22nd day after birth), increase in relative weight of liver and kidney to body weight, increase in sperm concentration, and histological change in liver and kidney were observed; and in F₁ females in the same group, decrease in luteinization hormone in serum (22nd day after birth), earlier opening of vagina, decrease in number of implanted parts, decrease in number of babies alive per litter, decrease in ovary weight and relative weight of ovary to body weight, histological change in liver, and decrease in mature body weight were observed. Effects on ovary tissues, reproductive ability, behavior and learning ability were not observed. The NOAEL relating to reproductive ability was estimated at 50 mg/kg/day, and the NOAEL relating to general toxicity and effect on next generation at 10 mg/kg/day.

(4) Other toxicities

Stated below are the reports on other toxicities as reported by the European Commission or Environment Canada/Health Canada. Reliability assessment is not made by MoE.

(a) Mutagenesis

- The European Commission reported the mutagenesis tests by Huls⁹⁴⁾, and Shimizu et al.⁹⁵⁾ using nonylphenol as test substance⁷⁾. As a result, nonylphenol was negative irrespective of existence of metabolic activation.

(b) Carcinogenesis

No report was obtained on carcinogenesis test using nonylphenol as test substance^{7, 17)}.

(c) Stimulus

- The European Commission reported that nonylphenol had dermal corrosiveness, eye stimulus, and respiratory stimulus⁷⁾.

(d) Oversensitivity

- The European Commission reported that nonylphenol had no dermal oversensitivity⁷⁾.

(e) Effects on human-derived cells

- Environment Canada/Health Canada reported damages to sperm, lymphocyte, and DNA of MCF-7 breast cancer cells as effects of nonylphenol on human-derived cells¹⁷⁾.

3. Literature search and reliability assessment about suspected endocrine disrupting effects, etc. on mammals

Stated below are the results of *in vitro* tests on endocrine disrupting effects of nonylphenol to mammals, as reported in the literature of 1966-2000 obtained through MEDLINE, etc., and in the reports by the Japanese Ministry of Economy, Trade and Industry, as well as by the European Commission or Environment Canada/Health Canada. Reliability assessment is not made by MoE.

- Nishihara et al.⁹⁶⁾ made a yeast two-hybrid assay using 4-nonylphenol (technical grade, isomer mixture, branched) and 4-*n*-nonylphenol (straight). As a result, 4-nonylphenol (branched) was positive ($REC_{10} 2 \cdot 10^{-7}$ M) and 4-*n*-nonylphenol was negative ($REC_{10} >10^{-3}$ M).
- Blair et al.⁹⁷⁾ made a rat uterus cytoplasm-derived estrogenic receptor binding assay using five kinds of 4-nonylphenol (isomer mixture, branched) and 4-*n*-nonylphenol (straight) from different manufacturers as test substances. As a result, IC_{50} of 4-nonylphenol (branched) ranged from 2.4 to 4.74 $\cdot 10^{-6}$ M, and IC_{50} of 4-*n*-nonylphenol was 2.80 $\cdot 10^{-5}$ M.
- Tabira et al.⁹⁸⁾ studied the response of Sf9 cells expressing human estrogenic receptor using nonylphenol (isomer mixture, branched) and 4-*n*-nonylphenol (straight) as test substances. As a result, IC_{50} of nonylphenol (isomer mixture, branched) was 3.7 $\cdot 10^{-6}$ M, and IC_{50} of 4-*n*-nonylphenol was 4.2 $\cdot 10^{-6}$ M.
- White et al.⁴⁹⁾ studied the effects on the human MCF-7 breast cancer cells exposed to 4-nonylphenol (4-NP, branched, according to the authors) at 10^{-7} , 10^{-6} , or 10^{-5} M. As a result, proliferation was observed at 10^{-5} M.
- Legler et al.⁹⁹⁾ made a human T47D breast cancer cell-derived estrogen receptor reporter gene assay using 4-nonylphenol (purity: 92.7%, according to manufacturer's catalog) as test substance. As a result, activity was observed, and EC_{50} was 2.6 $\cdot 10^{-7}$ M.
- Balaguer et al.¹⁰⁰⁾ studied the response of human breast cancer cell-derived MCF-7 and Hela cells with estrogen receptor reporter gene using nonylphenol (purity: 90%, isomer mixture, branched) and 4-*n*-nonylphenol (straight) as test substances. As a result, both nonylphenol (isomer mixture,

branched) and 4-*n*-nonylphenol showed estrogenic activity.

- Jorgensen et al.¹⁰¹⁾ studied the expression of endogeneous estrogen response gene in the human breast cancer cell-derived MCF-7 cell using nonylphenol (technical grade) as test substance. As a result, nonylphenol showed gene induction at almost the same level with genistein, a vegetable estrogen.