Fate of estrogens in sewage treatment process and effect of treated sewage on fish

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Abstract: Estrogens in treated sewage are suspected to pose an endocrine disrupting effect on fish, but it is becoming clear that most of the estrogens can be removed in a sewage treatment process. However, the knowledge about the relation between operation conditions and removal efficiency and the accurate fate in a sewage treatment process are restricted. Moreover, it cannot be said that sufficient information has been obtained about the effect of treated sewage on fish. This paper reports the results of a review of the relationship between MLDO concentration and estrogen removal in aeration tanks of activated sludge process, and the biological impact of treated sewage. The batch experiment where MLDO is controlled was conducted. Although no major change in E1 was observed when MLDO was still at a low level, it was confirmed that E1 concentration suddenly dropped when MLDO reached 3 mg/L. The rate of E1 reduction is speculated to depend closely on the concentration of dissolved oxygen. The exposure of medaka to treated sewage lead to the clarification of the relationship between estrogenic activity and the vitellogenin concentration in the sample water. It was also confirmed that the higher estrogenic activity, the higher the rise seen in vitellogenin concentration.

Keywords: estrogens, estrone, 17β-estradiol, feminization of fish, medaka,

Introduction

“Our Stolen Future,” authored by Dr. Theo Colborn et al., was translated into Japanese and published in September 1997. This book brought the problem of endocrine disruptors (EDs), popularly called environmental hormones, to public notice in Japan. Since then, a variety of investigations and surveys have been conducted in the field of sewage treatment. Since environmental hormones are known to be able to affect life at even extremely low concentrations, they have drawn a very high level of public concern. The Ministry of Land, Infrastructure and Transport (MLIT), as well as local governments and research institutes, have engaged in active research from the viewpoint of preventing any impact on human health and the ecosystem.

Following an investigation of 47 sewage treatment plants conducted jointly by MLIT and local governments, a report was issued noting that levels of environmental hormones in sewage influent were greatly decreased during the sewage treatment process, and many substances decreased by over 90%,. Estrogens originating in humans and animals (17β-estradiol or E2 and estrone or E1) showed a steep drop in levels in discharged water. Most of those substances were in the form of estrones [MLIT, 2001]. However, there is a conspicuous lack knowledge of the details of the behavior of estrogens during the sewage treatment process and the relationship between the operating conditions of the plant and the removal efficiency. To help sewage treatment plants adopt specific strategies to minimize outflow of these trace chemicals in the future, it is necessary to accumulate the basic information required to help predict and evaluate the impact of the operational conditions of the sewage treatment plant on the discharge of these chemicals to the environment.

The numerous reports have been issued in Europe and North America on anomalies in reproductive organs of wildlife or their abnormal reproductive conduct have propelled the environmental hormone issue to the top of the environmental agenda [e. g., Jobling et al., 1996; Routledge et al., 1998; Gray et al., 1999; Metcalfe et al., 2001; Jones et al., 2003]. Some of the phenomena reported include the feminization of male river fish living near the discharge ports of sewage treatment plants, with hermaphrodite fish observed to have eggs inside their testes [Routledge et al., 1998]. The causes of these phenomena are probably disturbance of the fish endocrine system by chemicals emitted as a result of human activity. The chemicals that predominantly cause feminization are reported to be estrogens, including E2 and E1, and a synthetic estrogen, 17α-ethynylestradiol or, EE2. However, there are fewer reports in Japan on the biological impact of treated sewage and sufficient findings have not been obtained. In urban areas, the ratio of treated sewage in the flow of rivers to which sewage treatment plants discharge water is increasing, making it urgently necessary to clarify the biological impact of treated sewage.

This paper reports the results of a review of the relationship between MLDO concentration and estrogen removal in aeration tanks of activated sludge process, and the biological impact of treated sewage.

Estrogen-like Activity in Sewage Influent, Treated Sewage and River water

The EDs that most noticeably cause feminization are called estrogen-like substances, since they cause conditions similar to those generally resulting from the presence of estrogen inside the body. They can be measured as estrogenic activity (17β-estradiol or E2 activity equivalent) using DNA recombinant yeasts with estrogen receptor genes. In our experiments, estrogenic activity was measured using a DNA recombinant yeast stock provided by Prof. John Sumpter of Brunel University, UK. The DNA recombinant yeast is designed to generate β-galactosidase when estrogens or estrogen-like substances bond with estrogen receptors (ERs). For the measurement of estrogenic activity using the DNA recombinant yeast method, we followed the method developed by Yakou et al. [Yakou, 1999], itself an improved version of the method of Routledge et al.

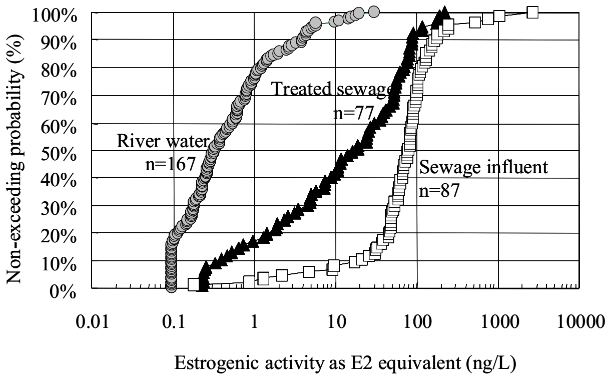

Figure 1: Concentration distribution of estrogenic activity in sewage influent, treated sewage and river water

Figure 1 shows the distribution of estrogenic activity concentration in sewage treatment plants and rivers in Japan. About 80% of estrogenic activity was removed in the treatment processes of sewage treatment plants on average; however, the treated sewage still carries a concentration of 16 ng/l-E2 activity equivalent (medium) on discharge to the environment. This concentration is a hundred times higher than the median concentration in rivers, which predicts a rise in estrogenic activity if the ratio of treated sewage to the entire flow of a river increases.

Theoretical activity was calculated from the concentrations of E1, E2 and nonylphenol (NP) obtained from chemical analysis of the sewage influent and treated sewage of 25 sewage treatment plants. We studied to what extent these substances contributed to estrogenic activity as obtained using the DNA recombinant yeast method. The estrogenic activity at each treatment plant and the corresponding theoretical values of E1, E2 and NP are shown in Figure 2.
Although there are numerous samples that can be explained by the presence of these three substances, many still defy such explanation, with the ratio between them varying according to treatment plant. It was also found that more than half of the 25 samples taken from both the sewage influent and treated sewage had a high content of estrogen-like active substances that cannot be traced to these three substances alone. To ascertain the contribution of each such substance, although there are some samples in the sewage influent that contain E1, E2 and NP each at certain levels, we took numerous samples from treated sewage that had a high E1 content. It was found that E1 is an important contributory substance for the estrogenic activity seen in treated sewage.

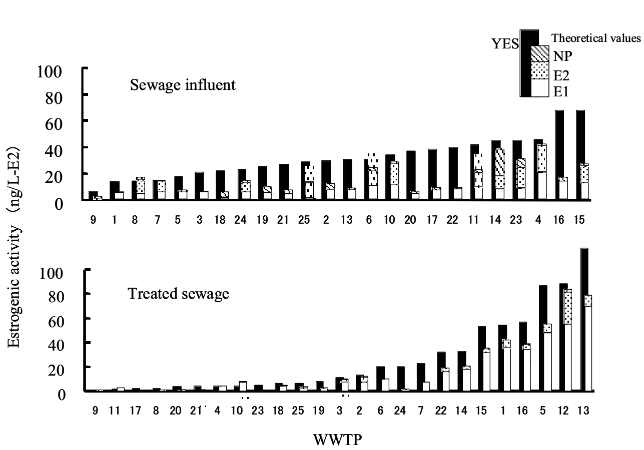

Figure 2: Contribution of each substance to estrogenic activity

Investigation of Behavior of Estrogen in Sewage Treatment Plants

Investigation method

We investigated a sewage treatment plant that serves 30,500 people with a conventional activated sludge process with a daily capacity of 20,400 m^3 in January and July 2004. For both investigations, samples were taken from each treatment process every 2 hours over 24 hours, and composite samples over 24 hours for flow proportion were prepared. The samples were analyzed for E2, E1, nonylphenol, estrogenic activity as well as the items listed in environmental quality standards related to the preservation of the living environment, such as SS and BOD.

Results and discussion

The samples from both dates show a good removal efficiency (over 90%) for SS, BOD and COD, but the removal ratio of T-N turned out to be low for the winter samples (16% in winter as opposed to 41% in summer). The samples from both seasons had a high removal efficiency for E2 (70% in winter and 87% in summer), whereas the concentration of E1 increased as treatment proceeded (740% up in winter and 50% up in summer) with this trend particularly prominent in winter. For estrogenic activity (Fig. 3), the activity level for the winter investigation rose as the treatment proceeded (removal efficiency of –97% in winter, but 39% in summer).

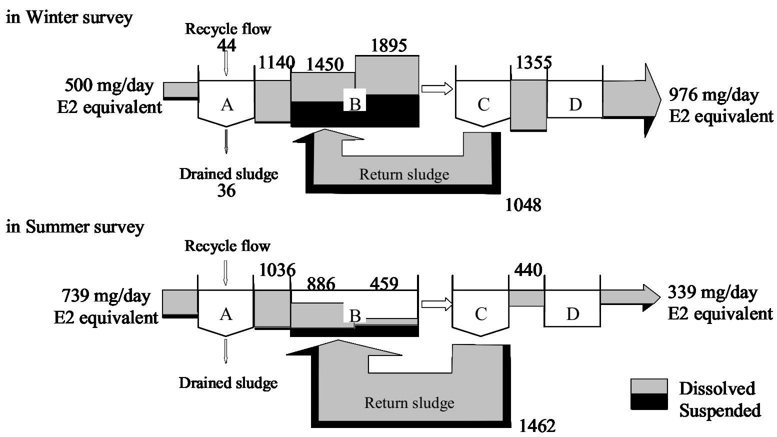

Figure 3: Mass balance of estrogenic activity (a, in mg/day, E2equivalent) through physicochemical treatment (A and C), biological treatment (B), and chlorination process (D) during winter and summer in a municipal sewage treatment plant in Japan.
A: Primary settling tank; B: Aeration tank; C: Final settling tank D: Chlorination tank

According to E1, E2 and NP concentration quantified by LC/MS/MS or HPLC analysis, the majority of estrogenic activity values detected in samples of each sewage treatment process were attributable to E1. In the summer, the plant was operated with a level of SRT and DO concentrations that promote nitrification, but in winter, the plant was operated at a reduced DO concentration in the aeration tank to reduce nitrification. These operation conditions may have affected the treatment of estrogen.

Relationship between MLDO concentration in the aeration tank and estrogen removal

As mentioned earlier, it is possible to speculate that removal of estrogen is affected by its relationship with the operational conditions of the sewage treatment plant (DO control). A batch experiment was therefore conducted to clarify the relationship between the MLDO concentration in the aeration tank and estrogen removal to find appropriate operation conditions that can stably reduce estrogen in the sewage during treatment.

Method

Analysis of the substances under study

Eight estrogens were to be investigated, including E2, E1, estriol (E3), and EE2 as well as sulfate conjugates of E2, E1 and E3, or estrone-3-sulfate (E1-S), β-estradiol-3-sulfate (E2-S), estriol-3-sulfate (E3-S), and estradiol 3, 17-disulfate (E2-diS). These substances were measured using the solid-phase extraction-LC/MS/MS method. The details of the analysis method are shown in the literature [Komori et al., 2004].

Investigation method

An aerobic batch experiment to simulate the treatment process in the plug flow type aeration tank (experiment duration: 8 hours) was conducted using the effluent from the primary settling tank and returned sludge to investigate changes in the levels of estrogen-related substances in the aeration tank of sewage treatment plant. The effluent from the primary settling tank and returned sludge were obtained from the conventional activated sludge process pilot plant (aeration tank 2 m^3 in capacity) using the sewage influent of the plant. The experimental equipment used is a 150-L stainless steel container fitted with an agitator, into which 55 L of the effluent from the primary settling tank and 25 L of returned sludge were fed and agitated. The water was aerated by air pumps through the air stones, and the aeration blow was adjusted to give an oxygen level of 3.0 mg/L by controlling the blow synchronously with the readings of the additionally fitted dissolved oxygen meter.

Results and discussion

Figure 4 shows temporal changes in pH, MLDO and estrogen in the aeration tank solution. When the experiment began, the dissolved oxygen supplied by aeration was initially rapidly consumed, leading to pseudo-anaerobic conditions. After one hour had elapsed, MLDO began to appear. It reached the set value of 3.0 mg/L in 7 hours. Levels of E2, which is a free estrogen, declined to below the detection limit under these pseudo-anaerobic conditions in 2 hours. There were no major changes in E1 until 6 hours after the start of the experiment. However, its concentration suddenly dropped when MLDO began to rise. As explained above, it is assumed that the reduction rate of E1 closely depends on the dissolved oxygen concentration. No EE2 was detected in any of the samples (detection limit: 0.5 ng/L). For the estrogen sulfate conjugates, E1-S and E3-S were lower than the level of free estrogen, while E2-S is almost equal to the free estrogen. E2-diS was detected at high concentrations. E1-S, E2-S and E3-S declined in the initial stages of the experiment, while E2-diS showed almost no change.

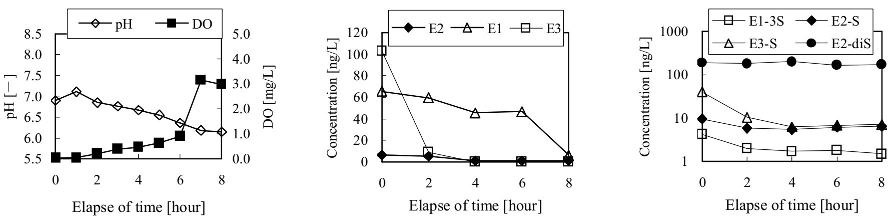

Figure 4: Temporal changes in pH, MLDO and estrogens

Effect of Treated Sewage on Fish

Effect of estrogen on Japanese medaka (Oryzias latipes)

An exposure experiment using adult male medaka in the laboratory was conducted with E2 and E1 as the substances under study to obtain basic knowledge on the effects of exposure to medaka of the treated sewage. Vitellogenin concentration in the livers of the sample medaka was measured to identify the minimum effective concentration and maximum ineffective concentration of E2 and E1 and the relationship between the exposure time and the concentration of vitellogenin.

Methods

Five test concentrations of E2 and E1 were used: 5.0, 12.6, 31.6, 79.5 and 200 ng/L. The sample medaka were about 5 months old, mature male medaka hatched from eggs taken from a group continuously raised in captivity for over three generations. The sample medaka were selected from a group of medaka that had been raised up to four months, to the maturing stage, as above, and were screened by sex based on their secondary sexual characteristic (shape of anal fin). Male fish alone were selected from the group and raised together until the experiment.

Sixty sample fish were used for each test section. They were exposed to the test water in flowing-water exposure equipment placed in a constant-temperature chamber lined with a stainless steel interior finish. The test water tank was made of hard glass with an effective water capacity of 10 L. The exposure time was 14 days.

Results and discussion

Figures 5 and 6 plot changes in vitellogenin concentration in the liver during the exposure time for E2 and E1. For vitellogenin in the control section, every individual was subjected to a concentration of lower than 1 ng/mg throughout the exposure period.

For E2, vitellogenin in the liver tends to rise in concentration in proportion to E2 concentration for each exposure period. On the 2nd and 7th day of exposure, no significant difference was observed between the measurement in the 5.0 ng/L section and that in the control section, but a significant difference appeared ($p < 0.01$) for the measurements on the 14th day of exposure.

For E1, no significant difference was observed at a standard of $p < 0.05$, in comparison with the control section in each exposure period for the below 31.6 ng/L concentration sections.

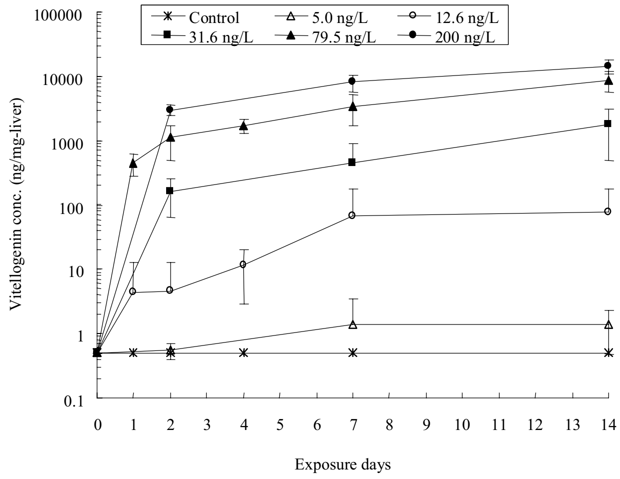

Figure 5: Temporal changes in vitellogenin concentration in the liver as result of exposure to E2

For the 79.5 ng/L and 200 ng/L sections, a significant difference ($p < 0.01$) was observed from the measurement in the control section on the 3rd, 7th and 14th day of exposure. Of the concentrations of vitellogenin in each exposure period, the lowest concentration (set concentration) that showed a significant difference ($p < 0.05$) from the control section is defined as the minimum effective concentration, while the highest concentration showing no significant difference is defined as the maximum ineffective concentration. The minimum effective concentration of E2 was 12.6 ng/L for the 2 day and 7 day exposure period and 5.0 ng/L for the 14 day period. The maximum ineffective concentration was 5.0 ng/L and <5.0 ng/L, respectively. The minimum effective concentration of E1 was 79.5 ng/L for the 3-day, 7-day and 14-day exposure periods, while the maximum ineffective concentration was 31.6 ng/L for all. Effective concentrations of 17β-estradiol and estrone are put together in a list as shown in Table 1.

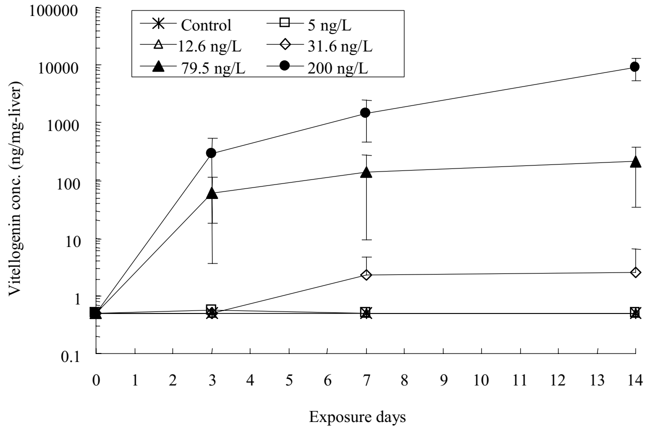

Figure 6: Temporal changes in vitellogenin concentration in the liver as a result of exposure E1

Table 1: Effective concentration of 17 β -estradiol and estrone

Substances under study	Exposure time (day)	Maximum ineffective concentration (ng/L)	Minimum effective concentration (ng/L)
17β-estradiol	2	5.0	12.6
	7	5.0	12.6
	14	< 5.0	5.0
Estrone	3	31.6	79.5
	7	31.6	79.5
	14	31.6	79.5

Impact on medaka of treated sewage

Methods

The influence of treated sewage on medaka was analyzed using the fish exposure test equipment placed in the sewage treatment plant. The equipment consists of three water tanks: the first tank removes sand and mud, the second tank maintains the water temperature at 25 ˚C, after which the water is led to the third tank (30 L) for exposure of medaka to the treated sewage.

The exposure test was conducted on adult medaka fish in treated sewage and sand-filtered water (Test A). Sixty male medaka and 10 female medaka were put in both the treated sewage section and the sand-filtered water section and exposed to the water for 6 weeks.

The other exposure test was conducted in treated sewage and sand-filtered water, on juvenile and freshly hatched medaka (Test B). Twenty freshly hatched fish were put in both the treated sewage section and the sand-filtered water section and exposed for 8 weeks. Estrogenic activity in the treated water and vitellogenin concentration in the medaka fish were measured during the test periods of both Test A and B.

Results and discussion

Changes in the level of estrogenic activity and the concentration of vitellogenin in the test are shown in Figure 7. In Test A, vitellogenin was found to have formed in the male medaka in the treated sewage section and the sand-filtered section. The level of estrogenic activity in both test sections was above 20 ng/L-E2 on the start of the test, but it subsequently never rose above 10 ng/L-E2. This fact suggests that the enhanced level of estrogen action seen at the start of the test was responsible for formation of vitellogenin in the medaka. The authors speculate that vitellogenin, once formed, will stay inside the body until metabolized. For Test B, male medaka produced vitellogen in the 8th week in the treated sewage section, where a rise in estrogenic activity beyond 20 ng/L-E2 in the 6th week was observed. As explained earlier, the concentration of estrogens, attributable to the formation of vitellogen in male medaka was 12.6 ng/L for the minimum effective concentration of E2 (2nd and 7th day of exposure), and the maximum ineffective concentrations were 5.0 ng/L (2nd and 7th day of exposure). Judging from this observation, it is possible that vitellogenin was formed as early as on the 2nd day of exposure when exposed to E2 at above 10 ng/L. This appears to be in agreement with the results of Tests A and B obtained from the experiment at the sewage treatment plant.

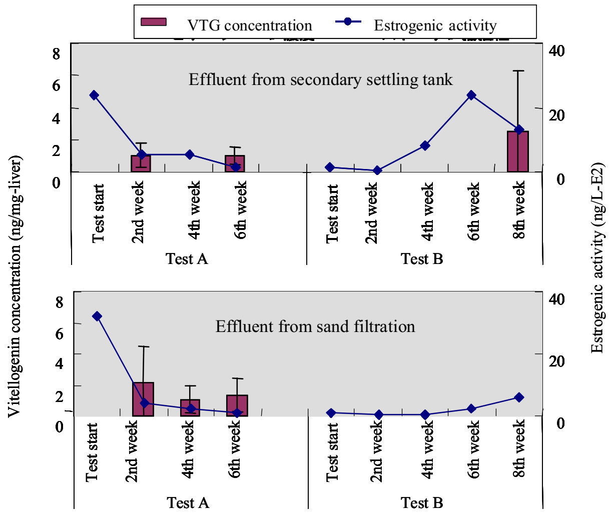

Figure 7: Changes in estrogenic activity and vitellogenin concentration in the experiment

The relationship between estrogenic activity and vitellogenin concentration in the sample water when medaka were exposed to the treated sewage is shown in Figure 8. Although there is a large variation, there is a tendency to show a higher estrogenic activity the more the vitellogenin concentration rises.

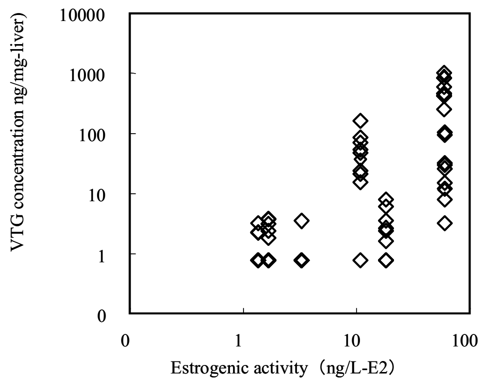

Figure 8: Relationship between estrogenic activity in the samples and VTG concentration in the livers of the mal medaka individuals in the exposure test

Conclusions

1) The concentration levels of estrogenic activity in the sewage influent, treated sewage and river water show that the level of estrogenic activity in the sewage decreases by about 80% during the treatment process at the sewage treatment plant, but the treated sewage containing a concentration of 16 ng/L-E2 activity equivalent (median) is discharged to the environmental water. This level is two digits higher than the concentration in the river water (median). As the ratio of treated sewage to the flow of a river rises, the level of estrogenic activity is predicted to rise.

2) Estrogenic activity in the sewage indicates that substances not traced back to E1, E2 and NP are contained in water samples. For the contribution of each substance, E1, E2 and NP are respectively responsible for raising the level of estrogenic activity in the sewage influent. It is thus confirmed that E1 has a high contributory role in the treated sewage and that E1 plays an important role as a major substance that enhances estrogenic activity in the treated sewage.

3) Behavior of estrogens in the sewage treatment plant was investigated in winter and summer. Although the removal ratio for E2 was found to be high in both seasons (70% for winter and 87% for summer), the concentration of E1 rises with the progress of treatment (740% up in winter and 50% up in summer), and this tendency is particularly conspicuous in winter.
4) The batch experiment where MLDO is controlled was conducted. Although no major change in E1 was observed when MLDO was still at a low level, it was confirmed that E1 concentration suddenly dropped when MLDO reached 3 mg/L. The rate of E1 reduction is speculated to depend closely on the concentration of dissolved oxygen.
5) The influence of estrogen on the formation of vitellogenin in male medaka was investigated. The maximum ineffective and minimum effective concentration of E2 in the 14-day exposure test were <5.0 ng/L and 5.0 ng/L, respectively. The maximum ineffective and minimum effective concentrations of E1 were 31.6 ng/L and 79.5 ng/L, respectively.
6) Male medaka fish were exposed to treated sewage and sand-filtered water. The results confirmed that vitellogenin had formed in the medaka in the treated sewage in which the estrogenic activity had risen above 20 ng/L-E2. The exposure of medaka to treated sewage lead to the clarification of the relationship between estrogenic activity and the vitellogenin concentration in the sample water. It was also confirmed that the higher estrogenic activity, the higher the rise seen in vitellogenin concentration.

Acknowledgment

The authors thank the local governments for kindly providing wastewater samples. We also thank the laboratory staff who prepared the samples for the estrogen-like activity and the LC/MS/MS analyses.

References

Gray, M. A.; Thather, K. L. and Metcalfe, C. D. (1999): Reproductive success and behavior of Japanese medaka (oryzias latipes) exposed to 4-tert-octylphenol, Environmental Toxicology and Chemistry (18) 11, 2587-2594

Jobling, S.; Sheahan, D.; Osborne, J. A.; Matthiessen, P. and Sumpter, J. P. (1996): Inhibition of testicular growth in rainbow trout (oncorhynchs mykiss) exposed to estrogenic alkylphenolic chemicals, Environmental Toxicology and Chemistry (15) 2, 194-202

Jones, E. T.; Thorpe, K.; Harrison, N.; Thomas, G; Morris, C.; Hutchinson, T,; Woodhead, S. and Tyler, C. (2003): Dynamics of estrogen biomarker responses in rainbow trout exposed to 17α-ethnylestrdiol, Environmental Toxicology and Chemistry (22) 12, 3001-3008

Komori, K.; Tanaka, H.; Okayasu, Y.; Yasojima, M. and Sato, C. (2004): Analysis and Occurrence of Estrogen in Wastewater in Japan, Water Science & Technology (50) 5, 93-100

Metcalfe, C. D.; Metcalfe, T. L.; Kiparissis, Y.; Koenig, B. G; Khan, C.; Hughes, R. J.; Croley, T. R.; March, R. E. and Potter, T. (2001): Estrogenic potency of chemicals detected in sewage treatment plant effluents as determind by in vivo assays with Japanese medaka (oryzias latipes), Environmental Toxicology and Chemistry (20) 2, 297-308

Ministry of Land, Infrastructure and Transport, MLIT, (2001): FY2000 Results of a Fact-finding Study of Endocrine Disruptors in Water Environments, Ministry of Land, Infrastructure and Transport (in Japanese).

Routledge, E.J.; Sheahan, D.; Desbrow, C.; Brighty, G C.; Waldock, M. and Sumpter, J. P. (1998): Identification of estrogenic chemicals in STW effluent. 2. in vivo responses in Trout and Roach, Environmental Science & Technology, 32, 1559-1565

Yakou, Y.; Takahashi, A.; Higashitani, T. and Tanaka, H. (1999): Measurement of Estrogen-like Activity in Wastewater Using Recombinant Yeast, Environmental Engineering Research, 36, 199-208, Japan Society of Civil Engineering, (in Japanese).

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Outline of the presentation

- ✓ Introduction and Objectives
- ✓ Estrogn-like activity in sewage influent, treated sewage and river water
- ✓ Investigation of behavior of estrogen in sewage treatment plants
- ✓ Relationship between MLDO concentration in the aeration tank and estrogen removal
- ✓ Effect of treated sewage on fish
- ✓ Conclusion

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3

"Our Stolen Future"

by
Theo Colborn,
Dianne Dumanoski
and
John Peterson Myers

translated by
Tsutomu Nagasaka

"Our Stolen Future" was translated into Japanese and published in September 1997

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Concentration of environmental hormones in sewage influent and treated sewage [1)]

Substances		Median concentration (μg/L) Sewage influent	Treated sewage	Removal efficiency
Nonylphenol (NP)		4.4	Trace (0.2)	95 %
Bisphenol-A		0.53	Trace (0.02)	96 %
2,4-dichlorophenol		0.07	N.D.	99 %
Diethyl phtalate		3.1	N.D.	99 %
Di-n-butyl phthalate		2.6	N.D.	99 %
Di (2-ethylhexyl) phthalate		12	Trace (0.4)	97 %
Di (2-ethylhexyl) adipate		0.09	N.D.	99 %
Benzophenone		0.17	0.05	71 %
17b-estradiol (E2)	ELISA	0.042	0.01	76 %
	LC/MS/MS	0.0081	N.D.	99 %
Estrone	LC/MS/MS	0.043	0.0064	85 %

1) MLIT, (2001): FY2000 Results of a Fact-finding Study of Endocrine Disruptors in Water Environments, Ministry of Land, Infrastructure and Transport (in Japanese)

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The report concerning the influences on wildlife [2)]

Wildlife		Place	Influences	Probable causative substances	Researchers
shellfish	reishia	seashores in Japan	virilescence, population	organotin components	Horiguchi et al. (1994)
fish	rainbow trout	rivers in the United Kingdom	virilescence, population	nonylphenol *not confirmed	Sumpter et al. (1985)
	roach (a kind of carp)	rivers in the United Kingdom	hermaphroditism	nonylphenol *not confirmed	Purdom et al. (1994)
	salmon	Great Lakes in the United States	thyroid hyperplasia, population	unknown	Leatherland (1992)
reptiles	crocodile	lakes in Florida State in the United States	male penis atrophy, decrease of hatchability of ovum, population	organochlorine agricultural chemicals such as DDT discharged into lakes	Guillette et al (1994)

Cf. Quoted works are based on "the interim reports by the workshop concerning exogenous endocrine-disrupting chemicals issues."

2) http://www.env.go.jp/en/chemi/ed/speed98/sp98t2.html

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Objectives

- A review of the relationship between MLDO concentration and estrogen removal in aeration tanks of activated sludge process
- The biological impact of treated sewage (Effect of treated sewage on fish)

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Outline of the presentation

- ✓ Introduction and Objectives
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- ✓ Conclusion

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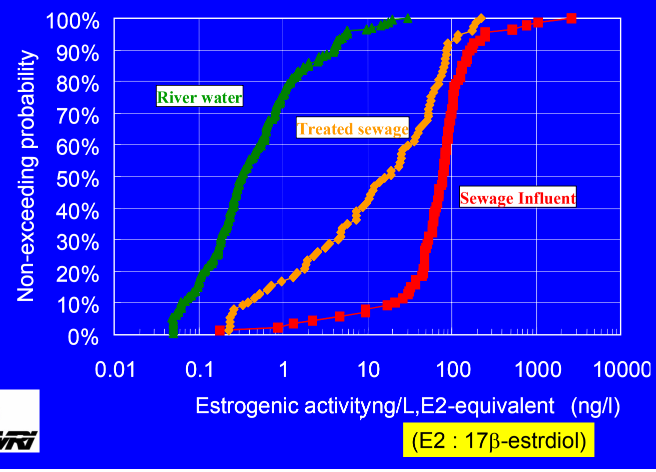
Concentration distribution of estrogenic activity in sewage influent, treated sewage and river water



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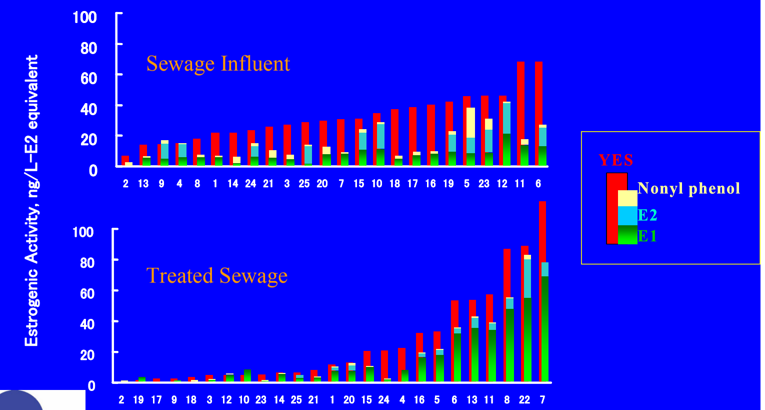
Contribution of Estrogens and Nonylphenol in Estrogenic Activity of Sewage Influent and Treated Sewage



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Outline of the presentation

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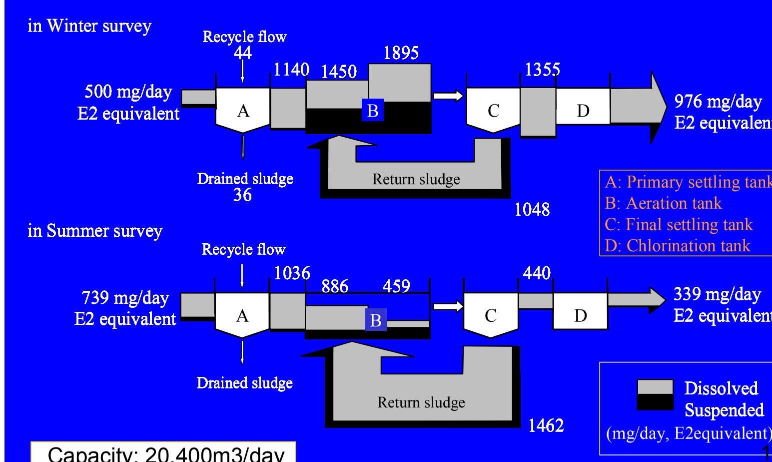
Mass balance of estrogenic activity in a municipal sewage treatment plant



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Outline of the presentation

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- ✓ Effect of treated sewage on fish
- ✓ Conclusion

FWRI

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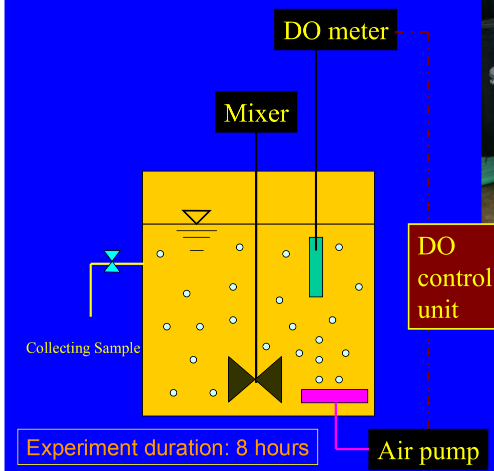

Analytical estrogens

E2, E1,E3, EE2, E1-S, E2-S, E3-S, E2-diS

Experimental apparatus

FWRI

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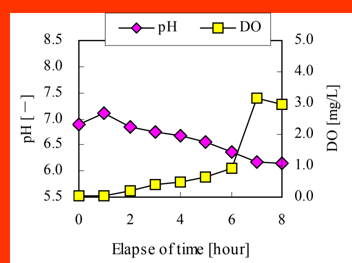
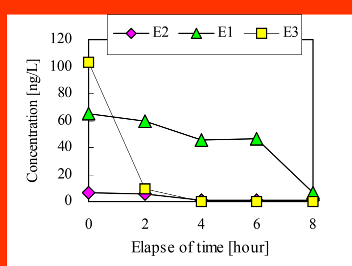
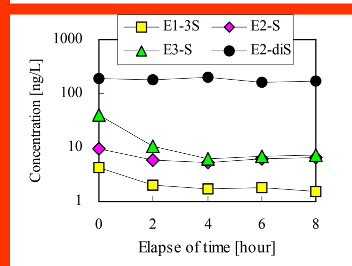
Temporal changes in pH, MLDO and estrogens







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Outline of the presentation

- ✓ Introduction and Objectives
- ✓ Estrogn-like activity in sewage influent, treated sewage and river water
- ✓ Investigation of behavior of estrogen in sewage treatment plants
- ✓ Relationship between MLDO concentration in the aeration tank and estrogen removal
- ✓ **Effect of treated sewage**
- ✓ Conclusion

FWRI

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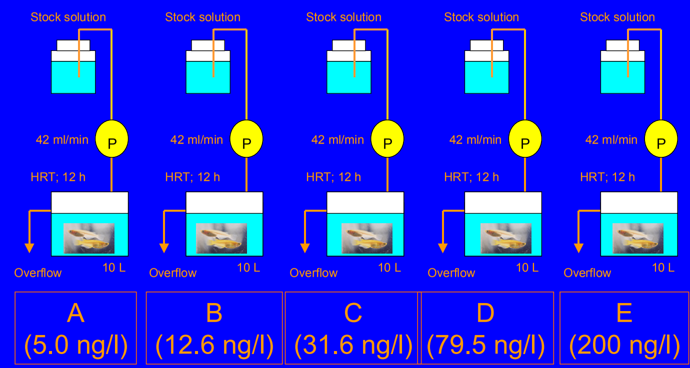
Effect of estrogen on Japanese medaka



- Test water: E2 & E1
- Sample fish: 60 for each tank
- Exposure time: 14 days

Vitellogenin concentration in the livers of the sample fish

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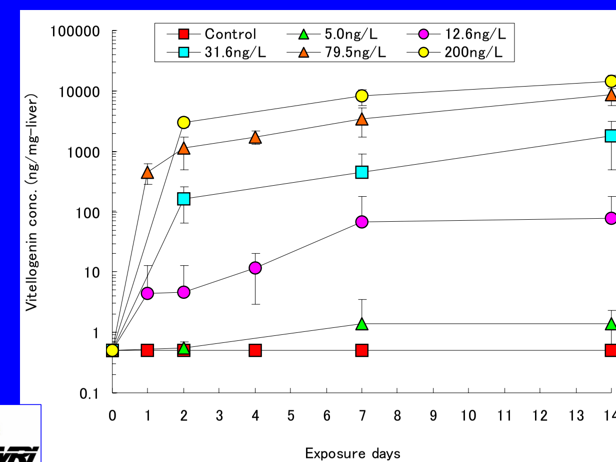
Temporal changes in vitellogenin (VTG) concentration in the liver as result of exposure to E2



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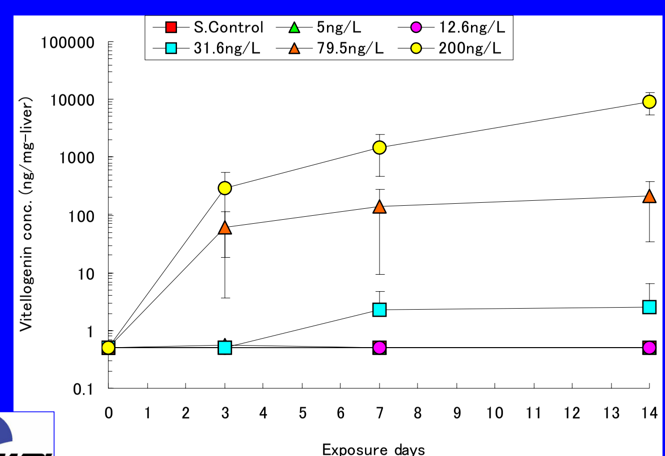
Temporal changes in vitellogenin (VTG) concentration in the liver as a result of exposure E1



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Effective concentration of E2 and E1

Substances under study	Exposure time (day)	Maximum ineffective concentration (ng/L)	Minimum effective concentration (ng/L)
E2	2	5.0	12.6
	7	5.0	12.6
	14	< 5.0	5.0
E1	3	31.6	79.5
	7	31.6	79.5
	14	31.6	79.5

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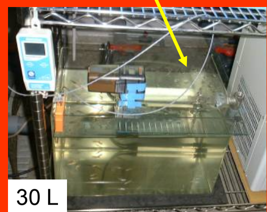
Impact on *medaka* of treated sewage

The first tank
(removes sand & mud)

The second tank
(maintains the water temperature)

The third tank
(fish exposure tank)



Test Water
- Treated sewage
- Sand filtered water

Test A; adult medaka fish

Test B; juvenile and freshly hatched medaka

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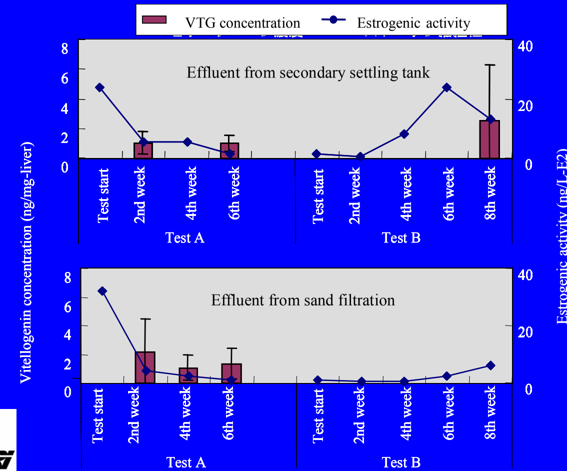
Changes in estrogenic activity and vitellogenin concentration in the experiment



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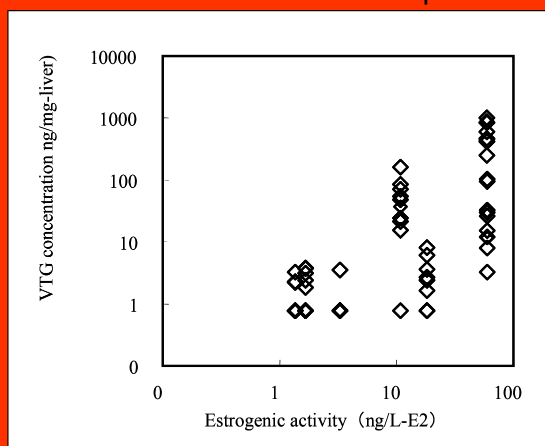
Relationship between estrogenic activity in the mples and VTG concentration in the livers of the mal *medaka* individuals in the exposure test



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Conclusions (1/3)

1) The batch experiment where MLDO is controlled was conducted

Although no major change in E1 was observed when MLDO was still at a low level, it was confirmed that E1 concentration suddenly dropped when MLDO reached 3 mg/L

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Conclusions (2/3)

2) The influence of estrogen on the formation of VTG in male medaka was investigated

	E2	E1
● Max. ineffective conc.;	<5.0 ng/L	31.6 ng/L
● Min. effective conc. ;	5.0 ng/L	79.5 ng/L

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Conclusions (3/3)

3) Male *medaka* fish were exposed to treated sewage and sand-filtered water

- The estrogenic activity had risen above 20 ng/L-E2
- The higher estrogenic activity, the higher the rise seen in VTG concentration

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Acknowledgment

The authors thank the local governments for kindly providing wastewater samples

We also thank the laboratory staff who prepared the samples for the estrogen-like activity and the LC/MS/MS analyses

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The latest research

- Development of analytical method for LVFX, CAM and AZM in sewage samples
- Occurrence of LVFX, CAM and AZM in sewage treatment plants in Japan

Collaboration with Towa Kagaku Co., Ltd.

- Analytical method of clarithromycin, erythromycin and its metabolite in waste waters

Collaboration with Teijin Eco-Science Ltd .

- Development of simultaneous analysis of pharmaceuticals in aqueous samples using LC-MS/MS

Collaboration with Murata Keisokuki Service Co., Ltd.
& Kyoto University

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10th Japanese German Workshop on Water Technology
Hotel Courtyard Marriott, Berlin
09.10-10.10.2006

Thank you for your attention

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