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The Estrogen Content and Relative Performance of Japanese and British Sewage Treatment Plants and their Potential Impact on Endocrine Disruption

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Key words: sewage treatment, estrogens, estradiol, estrone, ethinylestradiol, removal efficiency, endocrine disruption, UK, Japan

Both the UK and Japan are densely populated islands with relatively short rivers. Therefore, both countries are likely to be highly exposed to contaminants emanating from their human populations. This review considered how effective the different sewage treatment facilities of the two countries are at removing steroid estrogens from the effluent. The methods of estrogen analysis in sewage effluent, the number and importance of different sewage treatment types, and their apparent effectiveness at removing estrogens were all considered. In both countries the activated sludge treatment was dominant in terms of people served and water discharged. The analytical techniques used by those studying estrogen concentrations in effluents in both countries were broadly similar. Activated sludge plant (ASP) effluent in the UK typically contained around 2 ng/L E2 and 8 ng/L E1, whilst Japanese ASPs typically reported E2 as below detection, and 10 ng/L E1 in their effluents. When estrogenic bioassays were used in Japan they typically record an estrogenic potency of 10 ng/L E2 equivalents. Even taking into account EE2 (not found in Japanese effluents), the overall estrogenicity of British sewage effluents would appear to be the same as that of Japanese (around 10 ng/L E2 equivalents). This suggests that the ASPs serving the large urban communities would have effluent of similar estrogenic potencies in Japan and the UK. Less information is available about the more numerous biological (trickling) filter plants (BFP) in the UK and oxygen ditches (OD) in Japan which tend to serve smaller, more rural communities. The available data would suggest that the BFPs are significantly less efficient than the ODs at removing E1. This would suggest that in similar circumstances, British headwaters (where this STP type is often found) might be more at risk from endocrine disruption than their Japanese counterparts. Overall, the higher apparent incidence of endocrine disruption in British wild fish compared to Japanese cannot be attributed to differences in the efficiency of their respective STPs.

1. Introduction

When considering the likely impact of endocrine disruption in a country a number of factors come into play. These will include the sensitivity of the indigenous wild fish to the endocrine disrupting chemicals (EDCs), the amount of available dilution in the local rivers with respect to sewage effluent, and the amount of EDCs discharged by the sewage treatment plants (STPs). This review will focus on the

sewage treatment component of this equation. For domestic STPs the most estrogenic component of sewage effluent is considered to be the steroid estrogens.⁽¹⁻⁴⁾ Steroid estrogens are excreted in µg per day amounts by the individual human and the severity of endocrine disruption in fish in receiving waters has been shown to correlate with the size of the human population (and dilution) associated with the resident STP.^(5,6) Whilst the human population is the source of these compounds, the sewage treatment plant (STP) which handles the human waste can be considered as the gatekeeper. Thus, the steroid estrogen removal efficiency of the STP will play a major role in mediating the extent of endocrine disruption in the receiving waters. Both the UK and Japan are densely populated islands with rivers which on the whole do not offer the high dilution factors that are often found on large continents like North America.⁽⁷⁻¹⁰⁾ Therefore, the estrogen removal performance of their STPs is of significant interest to both countries. For the UK, the absence of measurements of estrogens and endocrine disruption in Scotland and Northern Ireland means that this review is necessarily focused on England and Wales. Although the survey of endocrine disruption in fish in rivers is still not as comprehensive as it might be, it would appear that fish are suffering more endocrine disruption in the UK than Japan.^(6,11,12) This review set out to examine whether the higher incidence of endocrine disruption in British waters was due to less efficient estrogen removal in British STPs than in Japanese STPs.

2. Comparisons

2.1 Type of sewage treatment employed in the two countries

2.1.1 England and Wales

Sewage treatment in England and Wales is managed by several private companies each with a regional base. The price that these private companies can charge the consumer is regulated by a Government body called the Water Services Regulation Authority (Ofwat). As part of this negotiation, information is exchanged between the companies and Ofwat. These include the number and type of STPs that the company possesses. The Ofwat classification is primarily a tool to allow analysis of a water service company's financial performance as part of the 'June returns, Chapter 17'. However, the classification is also suitable for discriminating simply between different treatment processes in terms of pollutant removal efficiency. The 2005 scheme (Table 1.) uses one of seven categories to classify all sewage treatment plants. It should be noted that where an STP load is split into two treatment streams, the works would be reported as the higher of the two proportions. For example, an STP with a split of 60% Secondary Activated Sludge and 40% Secondary Biological, would be classed as Secondary Activated Sludge (SAS). Where an STP comprises tertiary treatment in both categories 1 and 2 the works should be classified as 2 (Table 1). The data refers to sewage outlets, so where an STP may have two outlets this may mean a double accounting error could occur. The number and type of STPs according to this Ofwat classification has been made available to CEH as part of a National Risk Assessment for the Environment Agency of England and Wales (EA). For the purposes of this review some of the Ofwat categories were amalgamated as shown in Table 2. The resident human population, as distinguished from total population

equivalent, was provided for each STP. The consented dry weather flow (DWF) is the upper limit that a company can discharge, and is thus likely to be an overestimate of true DWF.

In this exercise 6,047 STPs have been classified, receiving waste from 51.4 million people, and discharging 14.3 million m³/d sewage effluent. The no biological treatment class represent only a small proportion of the total outfalls (Table 3), and serve only a very small percentage of the population (Table 3). Simple BFP type plants are the most numerous plants in England and Wales, however they serve less than 20% of the population (Table 3). The different ASP types are less numerous but they are the most important in terms of population served, and sewage effluent generated (Table 3). The BFP group with tertiary treatment is as important in terms of people served and effluent generated as the simple BFP group (Table 3).

2.1.2 *Japan*

Sewerage systems in Japan are managed by municipalities, except that very large sewerage systems covering several municipalities are managed by prefectural governments. Construction of sewerage system started in big cities about 100 years ago for the prevention of epidemic disease. After 1970 the sewer and STP system was established in law as the critical facility to control water pollution. After the coverage of sewerage system in major cities, the construction of sewerage systems in outlying towns and villages increased, particularly from the middle of the 1980's.

Like the UK, in Japan the major treatment process is activated sludge with a few biofilm processes (Table 4). Unlike the UK, where the BFP approach is very common, the major sewage treatment process for smaller scale facilities is the oxidation ditch (OD). This has been favoured because the long HRT and low maintenance requirement of the OD process are regarded as the ideal combination of features for the smaller scale STP. The bigger cities located in bay areas (such as Tokyo, and Osaka) are now introducing nutrient removal processes with longer hydraulic retention times (HRT) to prevent eutrophication of the bays.

Data is available from a review carried out by the Japan Sewage Works Association in which 1,921 STPs in operation all over Japan have been classified, receiving waste from 75 million Japanese residents (about two thirds of the total population) and discharging a total sewage flow of 55 million m³/d.⁽¹³⁾ Of this 24% of the capacity is discharged to sea, leaving 76% (42 million m³/d) of the sewage flow to be discharged into the inland rivers.

2.2 *Comparison of estrogen sampling and analytical methods*

2.2.1 *British methods*

The removal rates calculated for two British STPs quoted in Johnson et al.⁽¹⁴⁾ were based on data first reported in Desbrow et al.⁽¹⁾. The objective of this work had been to follow a toxicity identification and evaluation approach to determine the most oestrogenic components of sewage effluent. In 1995 two to three separate grab samples from the STPs were taken, filtered and extracted on a C18 SPE. The samples were cleaned using high performance liquid chromatography (HPLC) fractionation using a Spherisorb ODS2 C18 column with a 40-100% methanol gradient. Analysis was by gas chromatography mass spectrometry (GC-MS) with the ion trap

spectrometer run in the electron impact ionisation mode. The methodology employed by Williams et al. ⁽⁷⁾ began with a single grab sample from the effluent each day for two weeks which was individually analysed. The 5 L samples were filtered (0.7 µm) and spiked with deuterated internal surrogates. The samples were extracted with a C18 cartridge, prior to elution with 85% methanol. The particulate fraction was extracted with dichloro methane (DCM) and combined with the aqueous extract which was further concentrated through evaporation. The sample was cleaned using an HPLC fraction collector using a Waters Spherisorb column with a gradient of methanol eluent ranging from 40 to 100%. Following further concentration the sample was derivitized with a mixture of pyridine, N-(tert-butyltrimethylsilyl)-N-methyltrifluoroacetamide and bis(trimethylsilyl) trifluoroacetamide before analysis by GC-MS-MS. A similar GC-MS-MS method was described in Johnson et al. ⁽¹⁵⁾ except that two grab samples were collected around midday about 1 month apart. In this case the particulate phase was discarded as it was not considered as contributing significantly to the estrogen load. These methods are summarized in Table 5.

2.2.2 Japanese methods

The first approach used to measure estrogens in sewage, treated sewage and river water was an enzyme-linked immuno-solvent assay (ELISA) described in Nasu et al. ⁽¹⁶⁾ and Tanaka et al. ⁽¹⁷⁾. However, it is now considered these early ELISA kits over-estimated E2 in the effluent. Tanaka et al. ⁽¹⁷⁾ also used conventional LC-MS-MS analysis. Samples were first filtered (glass fibre) and the residue supersonically extracted with methanol. The filtrate was extracted with an Oasis HLB solid cartridge and eluted with methanol. Both the eluent and the supersonic extract were mixed, then the mixture was cleaned up through a florigel column and a thin-layer chromatogram. The samples were measured by LC-MS-MS-single ion monitoring using internal standards. Komori et al. ⁽¹⁸⁾ developed a modified method for analysing estrogens and their conjugates in wastewater. Sample preparation of this method consists of solid-phase extraction with an Oasis HLB cartridge for the filtrate, supersonic liquid extraction by methanol for suspended matter, and cleaning up with Sep-Pak Plus Florisil and Sep-Pak Plus NH₂. The cleaned-up sample was analyzed using a LC-MS-MS. Nakada et al. ⁽⁴⁾ developed a comprehensive fractionation method combined with GC-MS for quantifying free estrogens with recombinant yeast assay for detecting estrogenic activity. This method of E1 and E2 measurement employed solid phase extraction and Soxhlet-extraction for filtrate and particulate fraction of samples, respectively, and purification using column chromatography, followed by determination on capillary GC-MS. Ohiwa et al. ⁽¹⁹⁾ developed more sensitive silyl derivatized method in combination with a high resolution gas chromatography/double-focusing mass spectrometry (HR-GC-MS). The four free estrogenic compounds were derivatized by commercially available silylation agents. The silylation reaction was fast for all the four estrogens, and a method using N,O-bis(trimethylsilyl)acetamide for trimethylsilyl derivatization that resulted in easy post-reaction sample concentration was selected. The instrument detection limit (IDL) of the HR-GC-MS method ranged from 0.2 to 0.5 pg, which is one order magnitude lower than that of the LC-MS-MS method. ⁽¹⁸⁾ This method and the LC-MS-MS method were compared for real sewage samples, and gave very similar values. These methods are summarized in Table 6.

3. Estrogen removal performances

3.1 British scenario

Because of the difficulties in assessing inter-conversion between E2 and E1, as well as deconjugation within a STP it is difficult to use the word 'removal' for individual steroid estrogens in sewage treatment.^(20,21) Therefore, we should use the word apparent removal. In general, British ASPs are capable of apparently removing 80-90% plus of the natural estrogens that enter the plants (Table 7), with a typical effluent containing 2.4 ng/L E2 and 7.8 ng/L E1. A greater variation in performance with E1 is observed than with E2. A similar performance and pattern is observed with the BFPs with tertiary polishing steps. However, the BFP group is noticeably worse at removing E1 than the other STP types (Table 7). EE2 is detected on occasions in effluent at very low concentrations, which makes it difficult to assess removal rates for this molecule. The Environment Agency of England and Wales⁽²²⁾ proposed a method for assessing the estrogenic potency for a mixture of steroid estrogens as estradiol equivalents (E2 eq) which was calculated as follows:

$$\text{E2 eq} = \text{E2} + \text{EE2} * 10 + \text{E1} / 3$$

Using the same potency equation with the combined UK measured values for ASPs (Table 7) the typical calculated effluent E2 eq value would be 10 ng/L of which E2 composed 24% of the total potency, E1 26%, and EE2 50%. The values for the BFP with tertiary treatment are virtually identical to that of the ASPs (Table 7) implying EE2 is the single most important estrogenic component of the major British sewage effluents as has been suggested by others.^(8,23) With the straight BFP group reviewed by Johnson et al⁽¹⁵⁾ the typical calculated effluent E2 eq value would be 16.7 ng/L, i.e. it has a higher estrogenic potency than other treatment types and in this case E2 composed 16% of the total potency, E1 54%, and EE2 30%. From the limited data available, the BFP removal performance for the steroid estrogens is similar to the ASP, except in the case of E1 where it is considerably worse.

3.2 Japanese scenario

The E2 data measured by ELISA method in the early 2000 period are now considered as overestimates, and therefore, these data have not been included in this performance evaluation. From the five survey reports which investigated 139 STPs, Japanese ASPs are capable of apparently removing between 74 and more than 90% of the natural estrogens that enter the plants (Table 8). A typical ASP effluent not having detectable E2 (<0.5 ng/L) and 10 ng/L E1. EE2 has never been detected in any effluent by solid phase extraction LC-MS-MS or GC-MS method in Japan. The concentration of E1 in effluent was generally higher than that of E2 (Table 8). The median estrogenic potency as measured by yeast estrogen screen assay was 10 ng/L E2 eq. So far only one OD type of sewage treatment plant has been examined, but this appeared to remove E2 entirely, and have little E1 giving an overall estrogenic potency of only 1.5 ng/L E2 equiv in the effluent.⁽²⁴⁾

4. Discussion

In terms of people served and effluent discharged the ASP is the major method of sewage treatment in both countries. To measure steroid estrogens British and Japanese scientists have used similar extraction and cleaning methods. The

preservation methods and LODs were broadly similar, although the more recent Japanese methods appear to have greater sensitivity. Whilst many of the E1 and E2 values are comfortably above the LODs the same cannot be said for EE2, thus making assessment of this chemical extremely difficult. Overall, the natural steroid estrogen values reported in the UK and Japan effluents are similar to those found in other parts of the developed world.⁽²⁵⁻²⁹⁾

Unlike the UK, no EE2 has been detected so far in Japanese sewage effluent. This is to be expected since surveys have shown it to be rarely selected as a method of contraception and indeed it was only approved by the Japanese Ministry of Health as a contraceptive in 1999.⁽³⁰⁾ The 2005 United Nations survey of contraceptive use (www.unpopulation.org) reports only 2.3% of women in Japan aged between 15 and 49 as using either the inter-uterine device or contraceptive pill, compared to 22% of similar category women in the UK (pill only). It might have been expected that UK effluents would have had a greater (calculated) estrogenic potency than Japanese due to the presence of EE2, but this does not appear to be the case. Overall it would appear that the estrogenic potency of the ASP effluent of the two countries are almost the same, albeit comprised of slightly different ratios of the major steroid estrogens. However, more research is needed particularly into possible variations in estrogen removal efficiency with season. It should be noted that sewage effluent can contain other estrogenic components, such as 4, tert. Nonylphenol, which will contribute to the overall estrogenic potency.

The numerous British BFPs, commonly associated with less densely populated sites were less efficient than the ODs used in similar situations in Japan at removing E1.^(15,24) This would suggest that in similar circumstances British headwaters might be more at risk from endocrine disruption than Japanese.

This review would indicate that the apparently greater extent of endocrine disruption in UK rather than Japanese wild fish is not due to differences in sewage treatment efficiency, with the most common treatment plants having effluent of a similar estrogenic potency. Explanations are more likely to be found in the different sensitivities of the native wild fish, and differences in national geography and hydrology.

- ASP are by far the most important method of sewage treatment in terms of population served and effluent discharged both in the UK and Japan.
- The effluents of ASPs appear to have the same estrogenic potency between Japan and UK.
- EE2 can be found at sub ng/L concentrations in UK sewage effluents but not in Japanese effluents.
- OD sewage treatment apparently more efficient than BFP at removing steroid estrogens.
- Greater apparent endocrine disruption in wild fish observed in the UK compared to Japan is not due to less efficient sewage treatment.

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Table 1
Classification scheme in the UK for all STPs

Designation	Description
P (Primary)	Include works whose treatment methods are restricted to preliminary and primary treatment (screening, comminution, maceration, grit and detritus removal, pre-

	<p>aeration and grease removal, storm tanks, plus primary sedimentation, including where assisted by the addition of chemicals e.g., Clariflow).</p>
SAS (Secondary activated sludge)	<p>As primary, plus works whose treatment methods include activated sludge (including diffused air aeration, coarse bubble aeration, mechanical aeration, oxygen injection, submerged filters) and other equivalent techniques including deep shaft process, extended aeration (single, double and triple ditches) and biological aerated filters as secondary treatment.</p>
SB (Secondary biological):	<p>As primary, plus works whose treatment methods include rotating biological contactors and biological filtration (including conventional filtration, high rate filtration, alternating double filtration and double filtration), root zone treatment (where used as a secondary treatment stage).</p>
TA1 (Tertiary A1)	<p>Works with a secondary activated sludge process whose treatment methods also include prolonged settlement in conventional lagoons or raft lagoons, irrigation over grassland, constructed wetlands, root zone treatment (where used as a tertiary stage), drum filters, microstrainers, slow sand filters, tertiary nitrifying filters, wedge wire clarifiers or Clariflow installed in humus tanks, where used as a tertiary treatment stage.</p>
TA2 (Tertiary A2)	<p>Works with a secondary activated sludge process whose treatment methods also include rapid-gravity sand filters, moving bed filters, pressure filters, nutrient control using physico-chemical and biological methods, disinfection, hard COD and colour removal, where used as a tertiary treatment stage.</p>
TB1 (Tertiary B1)	<p>Works with a secondary stage biological process whose treatment methods also include prolonged settlement in conventional lagoons or raft lagoons, irrigation over grassland, constructed wetlands, root zone treatment (where used as a tertiary stage), drum filters, microstrainers, slow sand filters, tertiary nitrifying filters, wedge wire clarifiers or Clariflow installed in humus tanks, where used as a tertiary treatment stage.</p>
TB2 (Tertiary B2)	<p>Works with a secondary biological process whose treatment methods also include rapid gravity sand filters, moving bed filters, pressure filters, nutrient control using physico-chemical and biological methods, disinfection, hard COD and colour removal, where used as a tertiary treatment stage.</p>

Table 2
Amalgamation of Ofwat categories into similar groupings for this review

Grouping	Ofwat classes	Comment
No bio treat	No treatment, outfall and P	Outfall believed to mean discharge direct to sea. No treatment may mean discharge to an estuary. P for primary likely to represent some small inland hamlets
Simple BFP	SB	Biological filters, or rotating biological contactors. Note biological filters appear to be more common.
ASP all types	SAS, TA1, TA2	Activated sludge with, or without biological, or physical tertiary treatment
BFP plus tert	TB1, TB2	Biological filters, or rotating biological contactors with biological, or physical tertiary treatment

Table 3

Importance of the different sewage treatment types in England and Wales by number, populations served, and wastewater generated

Treatment	Number	% number	Human PE	% human PE	Consented DWF (m3/d)	% DWF
No bio treat	596	9.8	972,144	1.9	277,992	1.9
Simple BFP	2965	49	6,418,101	12.5	1,911,617	13.3
ASP all types	1147	19	37,232,688	72.5	10,321,846	72
BFP plus tert	1339	22.2	6,737,418	13.1	1,811,172	12.6
Total	6047	100	51,360,351	100	14,322,627	100

Table 4

Importance of different treatment types within a group of 1,921 STPs which treat two thirds of the Japanese population

Treatment	Number	% number	Human P	% human P	Consented DWF (m3/d)	% DWF
BFP	53	2.8	360,520	0.5	372,977	0.7
BAFP*	55	2.9	215,017	0.3	74,080	0.1
OD*¹	807	42.0	3,134,932	4.2	1,370,862	2.5
ASP	776	40.4	58,000,435	77.1	43,878,983	80.1
BFP plus tert*²	5	0.3	85,513	0.1	47,990	0.1
OD plus tert	49	2.6	153,102	0.2	78,690	0.1
ASP plus tert	60	3.1	3,775,552	5.0	2,807,081	5.1
BNR*³	116	6.0	9,511,259	12.6	6,174,402	11.3
Total	1921		75,236,330		54,805,065	

* Biological aerated filter plant

*¹ Oxidation Ditch

*² Tertiary treatment such as sand filtration

*³ Biological nutrient removal

Table 5
 Review of methods used to determine estrogens in UK sewage effluents

Reference	Sampling	Sample Preservation	Deuterated internal standards?	Extraction	HPLC fractionation cleaning?	Analysis	LOD (ng/L) for E1, E2, EE2
1	Composite	Chilled 4-6°C	No	C18 cartridge	Spherisorb ODS2 C18	GC-MS	'generally around 0.2 for all'
7	Separate grab samples	Chilled 4-6°C	Yes	C18 cartridge	Spherisorb S50DS1	GC-MS-MS	0.4-1.0, 0.6-1.0, 0.6-1.0
15	Separate grab samples	Chilled 4-6°C	Yes	C18 cartridge	Silica SPE column	GC-MS-MS.	0.4, 0.4, 0.5

Table 6
Review of methods used to determine estrogens in Japanese sewage effluents

Reference	Sampling	Sample preservation	Deuterated internal standards?	Extraction	HPLC fractionation cleaning?	Analysis	LOD (ng/L) for E1, E2, EE2
17	Separate grab samples/Composite sample	Chilled 4-6°C	Yes	Oasis HLB	a florigel column and a thin-layer chromatogram	LC-MS-MS	0.5(E1,,E2, EE2 by LC-MS-MS
18	Composite sample	Chilled 4-6°C	Yes	Oasis HLB	Sep-Pak Plus Florisil and Sep-Pak Plus NH ₂ .	LC-MS-MS	0.5(E1,,E2, EE2 by LC-MS-MS
4, 31	Grab samples/ Composite sample	Chilled 4-6°C	Yes	tC18/ Oasis HLB	5%-H ₂ O-deactivated silica gel column	GC-MS	0.05(E1) 0.03(E2)
19	Separate grab samples?	Chilled 4-6°C	Yes	Oasis HLB	Sep-Pak Plus Florisil	HR-GC-MS	0.04(E1,,E2, EE2 by LC-MS-MS

Table 7

Review of published data on steroid estrogens present in British sewage effluents

Reference	STP	Type	E2 ob. (ng/L)	E2 % removal	E1 ob. (ng/L)	E1 % removal	EE2 ob (ng/L)	E2 equiv (ng/L)
14	Rye Meads	ASP	4.5	71	3	95	ND*	
14	Deepham	ASP	8	40	8	85	ND	
14	Gt Billing	ASP	0.9	95	4.6	94	0.7	
15	ASP4	ASP	3.3	48	31	0	NQP* ²	
15	Three ASPs	ASP	0.5, 0.2, 0.2	95, 96.5, 99	7.5, 2, 0.2	89, 96, 99	<0.5-1	
ASP totals (n=8)		ASP	2.4 (SD 2.7)	79 (SD 23)	7.8 (SD 9.7)	81 (SD 33)	0.5 (SD 0.5)	10
15	Nine BFPs	BFP	2.7 (SD 5.9)	70 (SD 36)	27 (SD 28)	30 (SD 31)	0.5 (SD 0.7)	16.7
7	Harpenden	BFP& tert	1.3	89	6.3	88	<0.5-3.4	
15	Nine BFPs with tert.	BFP& tert	0.7 (SD 1)	89 (SD 6)	16.6 (SD 8.7)	74 (SD 29)	0.4 (SD 0.6)	
BFP & tert totals (n=10)			0.7 (SD 0.9)	89 (SD 6)	13.4 (SD 18)	76 (SD 28)	0.5 (SD 0.6)	10.2

*ND not detected

*²NQP non quantifiable peak

Table 8

Review of published data on steroid estrogens present in Japanese sewage effluents

Reference	STP	Type	E2 ob. (ng/L)	E2 % removal	E1 ob. (ng/L)	E1 % removal	Effluent E2 equiv (ng/L)	EE2 ob.
17	10-38 STP	ASP	ND(L)	>81	median 5.4(L)	87	median 1.8	ND(L)
18	20 STP	ASP	median ND(L)	>91	median 12(L)	47	median 4	ND(L)
4	1 STP	ASP	4.6, SD 3.0(G)	NA	33, SD 11(G)	NA	3.5, SD 2.5	NA
31	3 STP	ASP	6.3, SD 4.9(G)	90	47, SD 32(G)	86	22	NA
32	77 STP	ASP	NA	NA	NA	NA	median 16	NA
ASP as a whole		ASP	ND (H)	>90 (H)	10 (H)	74 (H)	10 (I)	ND
24	1 STP	OD	ND	95	median 4.4	85	1.5	ND

NA: not analyzed

ND: not detected

(L): values measured by Solid Phase Extraction LC-MS-MS Method

(G): values measured by Solid Phase Extraction GC-MS Method

(H): whole median estimated by LC/MS/MS data by Tanaka et al. (2003), Komori et al.(2004) and Nakada et al., (2004, and 2006)

(I) : whole median estimated by yeast estrogen screen assay data by Tanaka et al. (2003), Suzuki et al.(2006), and Nakada et al.(2004, and 2006)