Study on the Occurrence of EDCs and Organochlorine Residues
EDC および残留性有機塩素化合物の発生に関する研究

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1. Introduction

Synthetic estrogens, such as bisphenol A (BPA) and nonylphenol (NP); persistent organohalogen, and natural estrogens such as 17β-estradiol (E2), estrone (E1), estriol (E3); are among the most concerned chemicals that are known to disturb the ecosystem. E1, E2, E3, BPA and NP are mostly detected in the area close to sewage treatment plants (STP) and considered as endocrine disrupting chemicals (EDCs). So it is necessary to analyze EDCs in the STP. Organochlorine (OC) pesticides such as hexachlorocyclohexane (HCH), hexachlorobenzene (HCB) and 2,2-bis(p-chloro-phenyl)-1,1,1-richloro ethane (DDT) are the most well known to persist in the environment. The OC pesticide residues are suspected to still remains in the lake sediment through the years. Consequently, temporal trend analysis is necessary in the lake Kasumigaura that received OC pesticide runoff from agricultural field during the 1950s to 1970s. The temporal trend of the OC residues in the lake could be elucidated by analyzing the achieve samples, which in turn can help to predict the future trend.

Some OC pesticides were also previously used in Indonesia. Although the used of OC pesticides has been officially banned since 1970s, but it is still very difficult to control the illegal use of these pesticides, especially DDT. There were many reports concerning DDT accident and DDT residues in Java, residues in North Sumatra and also in some fruit and vegetables. Very high concentrations of DDT residues (ppm level) have also been found in soil samples, chicken tissues, fish tissues and spawn in 1986/1987. However, few studies have been done to reveal the present pollution status and to evaluate exposure in the country. Those facts lead this study to observe the present pollution status and to evaluate the exposure in the country.

2. Materials and methods

Two groups of EDCs samples were taken from a sewage treatment plant located near Tamagawa River, at which high EDCs activities have been found.

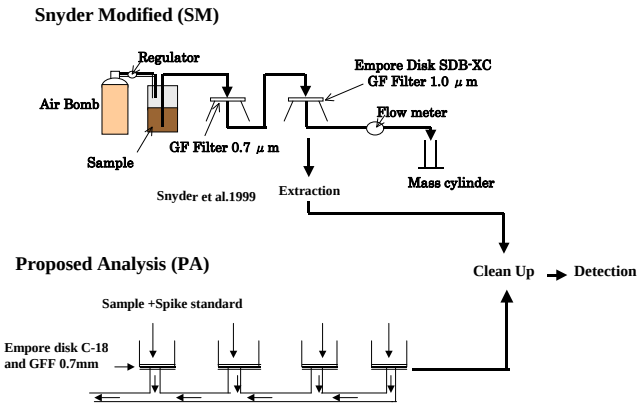

Figure 1. Diagram of pretreatment process for EDCs

Two pretreatment methods are proposed; a modification of Snyder method (SM method) and a proposed alternative method (PA method). The sample preparation by enrichment and extraction steps of SM method, were slightly modified from the method proposed by Snyder et al. (1999). The PA method was established during the study. The same clean up step were applied in both methods, and was inspired by the work of Ternes et al. (1999) , as shown in Figure 1. The samples were finally injected into HPLC-MS for measurement.

Biota samples from Lake Kasumigaura and Lake Mrica were selected in this study. Prawn, small fish and large fish samples were taken from lake Kasumigaura, Japan where OC pesticides has previously used in the basin. Small, medium, large fish shrimp and plankton were taken from lake Mrica Indonesia, where OC pesticides are suspected still to be consume illegally.

Prior to extraction, samples were decanted from formalin for formalin-preserved samples, or thawed for refrigerator preserved samples. They were then removed their moisture, grinded and homogenized. Moisture removal were performed using freeze-dried for samples from lake Kasumigaura, Japan and using sodium nitrate anhydrous for samples from lake Mrica, Indonesia. The known amount of powdered samples was extracted in a Soxhlet apparatus for 16-h using DCM. Labeled internal standards were spiked into hexane extracts of the samples prior to sulfuric acid treatment. Extracts were then passed through activated silica gel, alumina column and active carbon packed in a glass column sequently, as shown in Figure2. Finally the sample extracts were measured using a gas chromatography (GC-MS) for pesticides and a high-resolution GC interfaced with high-resolution MS (HRGC-HRMS) for dioxin and PCBs.

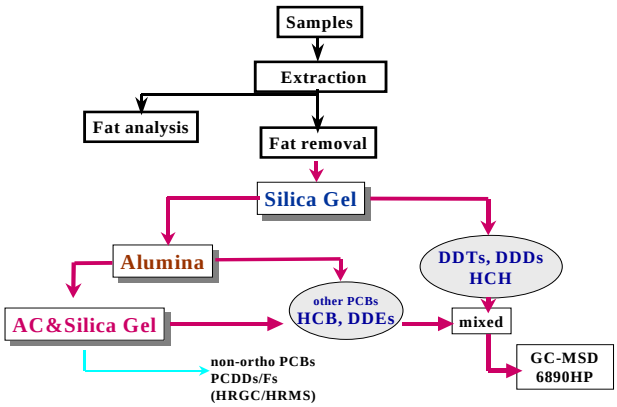

Figure 2. Diagram of pretreatment method for OC residues

3. Results and discussions

The concentrations of natural estrogens in the STP were significantly reduced to an undetectable level during the sewage treatment process. However, E1 and E2 levels were elevated significantly in the aeration. This elevation strengthened the

presumption of conjugate degradation in the aeration tank. Both E1 and E2 concentrations were decreased significantly by the end of STP process. The concentration of target EDCs in the STP are shown in Table 1.

Table 1. EDC concentrations in an STP (ng/ liter)

	Influent	Sedim. I	Aeration	Sedim II	Effluent
E1	12-16	1.7-15	10-17	0.67-8.7	<0.21-2.6
E2	12-20	5.9-25	13-29	<0.37-25	<0.41-13
E3	3.6-12	<0.58-13	3.0-7.6	1.4--15	1.2-6.3
BPA	2.6-15	<2.4-14	9.8-13	1.5-12	0.6-4.9
NP	< 2.3 #	<2.4 #	< 5.6 #	6.1	7.1

Note: #: undetectable value, (due to peak interferences)

The transformation among natural estrogens, besides the degradation of E2 conjugates may be some of the reason behind this elevation. The elevation of endocrine activities during the aeration process was confirmed to be consistent with those detected using bioassay procedure. These concentrations were generally lower than those reported in the literature, indicated that the wastewater seems to be well treated in the chosen STP. It can also be noted that the concentrations detected in the influent of STP are reasonable from the viewpoints of theoretical concentrations excreted by the population served by the STP. The theoretical concentrations estimated from the data found in the literatures, and were calculated by the following equation:

$$C = \frac{\sum(n_i X_i)}{V}$$ where:

C = estimated concentration (ng/l)
n_i = population that served by an STP (410000 persons)
X_i = estrogen excreted by each population
V = volume of waste water (175140 m^3/day)

The theoretical concentrations were generally higher than those detected in the real samples. It should be noted that the degradation process and conversion of natural estrogens also take place before entering the STP.

Concerning OC studies, the recovery percentage obtained for the prawn samples from lake Kasumigaura range between 64% and 117%. The recovery rates found in large fish and small fish samples ranged between 50 and 67% and ranged between 70 and 114%, respectively. The recovery rates for Mrica samples range between 57.6 and 156%. The moisture content of prawn samples and fish samples from lake Kasumigaura ranged from 73% to 82% and ranged from 57.0 to 83.3%, respectively. The moisture contents for large, medium, small fish, plankton and shrimp samples from lake Mrica were 77.9, 78.3, 79.2, 81.3 and 79.2%, respectively.

Beside the measurement of OC residues from some archive samples, the amount of two OC residues remaining in the lake is simulated in this study. The simulation was performed based on the amount of HCH and DDT pesticides sprayed in the basin by an exponential equation, modified from the first-order degradation reaction and runoff approached, as shown in the following equation.

$$Q(i) = C(i) + Q(i-1)e^{\left(\frac{0.693}{T_{1/2}}\right)}$$, where:

$Q(i)$: amount of OC residues remaining in the year i (ton)
$C(i)$: amount of OC pesticides sprayed in the year i (ton)
$T_{1/2}$: half-life of the OC in the soil of catchment area (year)

The necessary data for this simulation were taken from statistical sources. The temporal trends of pesticide loss by degradation and run-off were calculated as the difference amount remaining in the year (i) and those in year (i-1) or loss = Q(i) - Q(i-1). The pesticide loss plotted with the real concentration detected in the small fish and prawn samples shown in Figure 3 and Figure 4 were assuming $T_{1/2}$= 5 years.

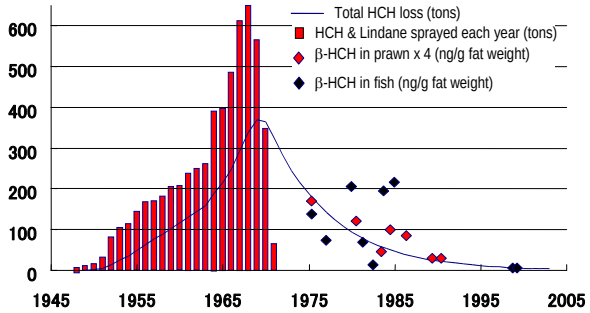

Figure 3. HCH sprayed, total loss and β-HCH residues

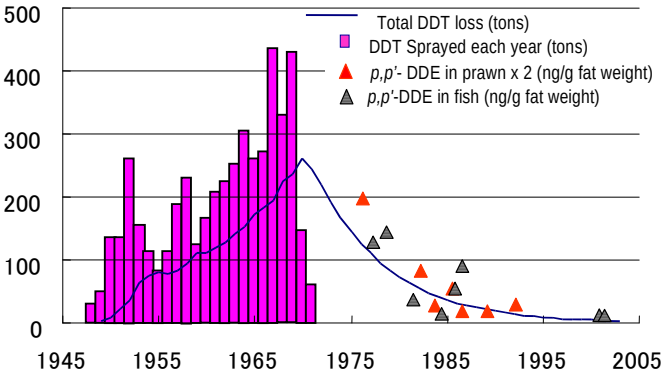

Figure 4. DDT sprayed, total loss and *p,p'*-DDE residues

The safety levels for Mrica samples were evaluated using simplified total diet study, as follows:

$$\frac{\left[\sum n_i \times c_i(fish) \times \text{fish consumed} + \sum DDT \text{from other food}\right]}{BW}$$

where c: concentration and BW : average body weight (60 kg)

The necessary concentrations were taken from current results, with the assumption data from literatures. The results were then evaluated using reference dose (RfD) calculated based on FAO/WHO data

4. Conclusions

The fluctuation of EDCs was observed during treatment process in the STP. The trends of β-HCH and *p,p'*-DDE residues could be explained by the effect of run-off HCH and DDT from the basin. The OC residues in Indonesia were considered as safe for normal consumption.

5. Related references

Sunardi et al., (2001), IJAS 1: 60-68; Sunardi et al., (2004), AECT (in press); Sunardi et al., (2004), TEC (submitted); Masunaga, et al., (2000), Env. Sci 7, 101-117; Senthil Kumar et al., (2004), TEC (in press)