BIOLOGICAL CONCENTRATION OF NONYLPHENOL AND BEHAVIOR OF NONYLPHENOL ISOMERS IN WATER ENVIRONMENTS

Yasunobu Kato[1], Tetsu Kumagai[2], Hiroaki Nagamitsu[1], Naoko Hiramatsu[2] and Yosohiro Sugie[1]

The Faculty of Engineering, Himeji Institute of Technology, 2167 Shosha Himeji-shi, Hyogo 670-2201 Japan[1] School of Humanities for Environmental Policy and Technology, Himeji Institute of Technology, 1-1-12 Shinzaike-honcho Himeji-shi, Hyogo 670-0092 Japan[2]

Introduction

Alkylphenols have been used as a raw material of non-ionic surfactants throughout the world, and these substances exist most widely in water environments[1]. Recently, these substances are known as endocrine disruptors. It was reported that Nonylphenol (NP) has isomers that have different ability as endocrine disrupters[2]. We collected water, plankton, blue mussel and black sea bream samples from Harimanada which is located in the Seto Inland Sea; Hyogo, Japan. NP and NP isomers in the all samples were determined by gas chromatography with tandem mass spectrometry. We discussed the behavior of nonylphenol and nonylphenol isomers in the food chain process in water environments.

Method and Materials

Materials and reagents The acetone, n-hexane, dichloromethane, methanol, ethanol, sodium chloride, potassium hydroxide, diethyl ether, diethyl sulfate, sodium sulfate, methyl acetate and ethanol suitable for pesticide residue · PCB grade. Sep-Pack PS-2 (Waters Co.) was use to concentrate water samples. Sep-Pak florisil (Waters Co.) was used to clean the samples. P-nonylphenol as a standard solution and phenanthren-d10 as surrogate substance were used.
Sampling Discharge of sewage treatment plant, river water, seawater, plankton, blue mussel and black sea bream were collected in wintertime 2000 and 2001 from the Himeji port of Harimanada. The Samples for nonylphenol were stored frozen at -20^{0}C in the freezer. Water samples were concentrated by Sep-Pack PS-2 and extracted by methyl acetate. The biological samples were homogenized with methanol and then centrifuged. The samples were added to hexane to remove

the lipids, including the reextraction of the dichloromethane, and concentrated to about 0.1mL.

Derivatization The samples added with 1mol/L-KOH/ethanol solution (0.5mL) and diethyl sulfate (0.2mL) were stabilized for 30minutes. Next, the samples with about 4mL 1mol/L-KOH/ethanol solution were held in 60^{0}C water bath for 60minutes and extracted by 1mL hexane containing 0.05ml internal standard to monitor fluctuations and correct the data accordingly. After the separation, the organic phases were corrected and cleaned up by Sep-Pak florisil with 10mL of 4%ether/hexane. The samples were concentrated to 0.5mL, except where high concentrations of NP were encountered before the gas chromatographic determination.

Determination of NP and NP isomers (Tandem mass) The final extracts were analyzed by gas chromatography/mass spectrometry (GC/MS-MS). The analysis was performed on a Finnigan GCQ equipped with an Xcalibur data system on a Gateway computer. The samples (2 μL) were injected spritless at 300^{0}C. The column was an Rtx-5MS (30m, 0.25mm i.d.). Helium was used as the carrier gas (30cm/min). The temperature program was 60^{0}C (1min)−10^{0}C /min−300^{0}C (10min). Mass spectrometric detection carried out by electron impact (EI) ionization on tandem mass spectrometry to monitor the ions. And, it was necessary to determine each sample in order to calculate the abundance ratio of each isomer in samples accuracy. Therefore, m/z=163 which the fragment-ion strength is strongest on representative 9 peaks was carried out by the processing of tandem mass, and the fragment-ion m/z=135 was monitored (Fig.1). The final real concentrations measured for the procedural blank were corrected by using the average concentration measured for the procedural branks and the recovery achieved from the spiked samples of all oligomers; recoveries of 80%-90% were obtained. Next, the abundance ratio of each isomer was calculated from the ratio between all peak areas and area of each peak. It was estimated that the structure of NP isomers is compared between the structure of each peak with NMR shift data (Bhatt et.al. 1992) and the analysis of the MS fragment ratio by Full scan.

Result and Discussion

It was clear that the enrichment factor of NP was about 1800 from seawater to the plankton by present measurement. For this reason, phytoplankton (Coscinodiscus, Eucampia zoodiacus, etc.) living in Himeji harbor in wintertime mainly have large surface areas, and hydrophobic substances like NP exist in high concentration on the surface of planktons. Therefore, enrichment factor 1.8 were low values in concentration moving from plankton to the blue mussel. However, when the concentration from seawater to blue mussel was considered, the enrichment factor was an almost equal value reported in a past paper[3] at about 3200 and 3400. And, it appeared that an enrichment factor almost 1.0 in the liver of the black bream did not occur in that the biological concentration

moving from the blue mussel to the black bream that eats it. Next, the behavior of nonylphenol isomers existed from discharge of water treatment plant to black bream was discussed on measurement of gas chromatography with tandem mass spectrometry. In this result, when we examined the NP isomers in the discharge of both sewage treatment plants, which had an one of which had an activated sludge process and the other had an activated sludge process and digested sludge process, two discharges have a different abundance ratio of NP isomers. The result suggests that discharge of two-sewage treatment plants were treated by different microorganisms lived in activated sludge process and digested sludge process. The changes on abundance ratio of NP isomers, which is located in the water from the discharge of sewage treatment plant to seawater, were discussed. It was reported that NP has isomers that have a different ability as endocrine disrupters. Especially, the 1,4 and 9 isomers have strong abilities as endocrine disruptors. In this study, it appeared that the 3,6 and 7 isomers tended to break down in water environments from the discharge of sewage treatment plant to seawater. But it means that other isomers included 1,4 and 9 isomers tend to stabilize in these environments. Thus there is concern about the effect on living things in water environments. On the other hand, it appeared that 1,4,7 and 8 NP isomers tend to exist stabilize in the food chain process (Fig.2). When the relation between the structure of NP isomers and the stability of NP isomers in water environments are compared, the structure in water is different from the structure in vivo. For this reason, it were thought that NP isomers in water tend to break down and NP isomers in vivo tend to change hydrophilic substances through the drug-metabolizing system.

Fig.1 Chromatogram and mass spectra of NP standard

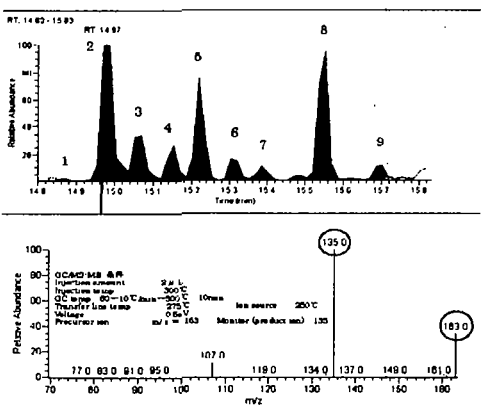
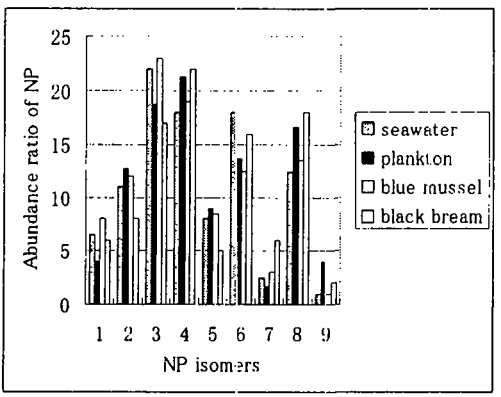


Fig.2 Abundance ratio of NP isomers in vivo



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