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4-Nonylphenol, 4-Nonylphenol Mono- and Diethoxylates, and Other 4-Alkylphenols in Water and Shellfish from Rivers Flowing into Lake Biwa

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Alkylphenolpolyethoxylates (APEOs) have been widely used in the last 50 years for a variety of industrial, household and commercial applications. Concern has increased recently about the wide usage of APEOs, because their biodegradation metabolites nonylphenol and octylphenol are stable and nonylphenol has been demonstrated to be toxic to both marine and freshwater species (Comber et al. 1993; McLeese et al. 1981), to induce estrogenic responses in male trout (Jobling and Sumpter, 1993; Purdom et al., 1994) and may accumulate in freshwater organisms (Ahel et al. 1993; Ekelund et al. 1990).

Surveys of nonylphenol and its ethoxylates in water samples of rivers, sewage effluents and estuaries have been widely performed in many countries (Ahel et al. 1994a; Ahel et al. 1994b; Kvestak et al. 1994; Naylor et al. 1992; Naylor 1992; Blackburn and Waldock 1995; Ahel et al. 1996; Bennie et al. 1997; Kojima and Watanabe 1998; Isobe et al. 1999). However, few reports (McLeese et al. 1981; Ekelund et al. 1990; Ahel et al. 1993; Wahlberg et al. 1990) have been published regarding contamination of fish, shellfish and other aquatic organisms by nonylphenol and its ethoxylates in the field.

We have already reported the surveys on 4-nonylphenols and 4-tert-octylphenol in water and fish from rivers flowing into Lake Biwa (Tsuda et al. 2000a). Bioconcentration potential of 4-nonylphenols and 4-tert-octylphenol in fish could be estimated from the field data. In this report, the same surveys were performed more extensively for 4-nonylphenol, 4-nonylphenol mono- and diethoxylates and other 4-alkylphenols in water and shellfish from two rivers flowing into Lake Biwa for the purpose of estimating bioconcentration potential of these chemicals in shellfish.

MATERIALS AND METHODS

4-Nonylphenol (NP), a mixture of compounds with branched side chains, 4-tert-butylphenol (BP), purity more than 98 %, 4-tert-octylphenol (OP), purity more than 93 %, and bisphenol A (BPA), purity more than 99 %, were purchased from Tokyo Chemical Industry (Tokyo,

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Japan). Nonylphenol monoethoxylate (NP1EO), purity 99.9 %, and nonylphenol diethoxylate (NP2EO), purity 99.5 %, were purchased from Hayashi Pure Chemical Ind. Co. Ltd. (Osaka, Japan). Sep-Pak Florisil (Waters, USA) and Florisil PR from Wako Pure Chemical Industries Ltd. (Osaka, Japan) after activation at 130℃ for 16 h were used for column clean-up of water and shellfish samples. HPLC-grade methanol and nano-pure-grade water were used for HPLC mobile phase. Pesticide-grade solvents and chemicals were used for sample preparations.

Water and shellfish samples were collected from the Fujima and Shiratori Rivers once every month from June 1999 to March 2000. The two rivers flow into Lake Biwa and are located in Ohmihachiman City in Shiga Prefecture. Shellfish samples were the river snail (*Senotaia quadrata histrica,* body weight 3.1 – 4.8g), and melanian snail (*Semisulcospira libertina libertina,* body weight 2.6 – 3.7 g). Water samples were analyzed immediately after collection. Shellfish samples were homogenized as a mixture of three or four samples (meat and viscera) for each sampling locationm, frozen and preserved for later analysis. Analyses were carried out from June 1999 to March 2000 for water samples and from June to October in 1999 for shellfish samples from the two rivers.

Each concentration of the chemicals in water samples was determined by the following procedure. A measured volume (500 mL) of water was shaken with 50 mL of dichloromethane after addition of 25 g of NaCl. The organic layer was filtered through anhydrous Na_2SO_4 and the aqueous layer was again shaken and filtered in the same manner. The combined filtrate was rotary-vacuum evaporated just to dryness at 40°C and the residue was dissolved in 1 mL of methanol. Determination of the chemicals in the methanol solution was performed by high performance liquid chromatography (HPLC) with a fluorescence detection (Tsuda et al. 2000b). Average recoveries (n=3) were 91% for BPA, 83% for BP, 89% for OP, 89% for NP, 91% for NP1EO, 98% for NP2EO at a spiked level of 1.0 ng/mL. Detection limits were 0.01 ng/mL for BPA, BP and OP, and 0.02 ng/mL for NP, NP1EO and NP2EO. Determination of the chemicals in shellfish samples was performed by the method of Tsuda et al. (2000b). In brief, shellfish sample (ca 5 g) was homogenized twice with each 30 mL of acetonitrile after addition of 5 g of anhydrous Na_2SO_4 and the organic layer was filtered through anhydrous Na_2SO_4.The combined filtrate was rotary-vacuum evaporated just to dryness at 40°C and the residue was dissolved in 10 mL of hexane. The hexane solution was shaken twice with each 30 mL of acetonitrile saturated with hexane and the acetonitrile layer was rotary-vacuum evaporated just to dryness at 40°C. The residue was dissolved in 5 mL of hexane and passed through a 30 cm × 1.0 cm ϕ glass clean-up column containing 5 g of hexane-rinsed Florisil PR and 1 g of anhydrous sodium sulfate. The column was washed with 50 mL of hexane, eluted with 80 mL of ethyl ether and hexane (1+9) for NP, BP and OP and with 40 mL of acetone and hexane (3+7) for BPA, NP1EO and NP2EO. Each eluate was rotary-vacuum evaporated nearly to dryness at 40℃,

transferred to a graduated test-tube (rinsing the flask with methanol) and adjusted to 1.0 mL under a stream of nitrogen at 40℃. The sample solution was analyzed by HPLC in the same manner as in the water sample. Average recoveries (meat and viscera of river snail 5 g, n=3) were 71% for BPA, 70% for BP, 80% for OP, 82% for NP, 85% for NP1EO, 90% for NP2EO at a spiked level of 100 ng/g. Detection limits were 0.5 ng/g for BPA, BP and OP, and 1.0 ng/g for NP, NP1EO and NP2EO.

Calculation of BCF

$$BCF = \frac{\text{chemical concentration in whole shellfish except shell}}{\text{chemical concentration in water}}$$

Calculation was performed at each sampling time when the concentration of each chemical could be determined for both water and shellfish samples.

RESULTS AND DISCUSSION

Results of the survey on water and shellfish samples in the two rivers are summarized in Table 1. For water samples, NP, NP1EO and NP2EO were detected all the year round at high frequency (59/60) in the two rivers. BPA, BP and OP were detected at lower concentrations and at lower frequency (16/60). Composition of NP, NP1EO and NP2EO was 21%, 45% and 34% (average, n=10) in Fujima River water and 19%, 55% and 26% (average, n=10) in Shiratori River water. NP concentrations in river water have already been reported in the other rivers flowing into Lake Biwa (Tsuda et al. 2000a). The present NP concentrations in the two rivers (ND ~ 0.30 ng/mL) from June1999 to March 2000 are lower than those of the other eight rivers (0.11 ~ 3.08 ng/mL) from April 1998 to March 1999. The difference is probably due to the sampling date not the sampling locations. That is, decreasing use of APEOs is probably occurring in Japan.

Table 1. Results of surveys on 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and other alkylphenols in water and shellfish samples from two rivers flowing into Lake Biwa

	Fujima River		Shiratori River	
Chemicals	Water (ng/mL)	River snail (ng/g, wet wt.)	Water (ng/mL)	Melaniansnail(ng/g, wet wt.)
NP	ND ~0.30 (n=10)	2.8~19.3 (n=5)	0.02 ~ 0.10 (n=10)	1.0 ~ 7.0 (n=5)
NP1EO	0.05~0.42 (n=10)	7.7~23.3 (n=5)	0.04 ~ 0.39 (n=10)	2.1 ~ 8.4 (n=5)
NP2EO	0.04~0.52 (n=10)	2.0 ~ 5.3 (n=5)	ND ~ 0.13 (n=10)	1.1 ~ 6.4 (n=5)
BPA	ND ~0.03 (n=10)	ND (n=5)	ND (n=10)	ND (n=5)
BP	ND ~0.10 (n=10)	ND (n=5)	ND ~ 0.02 (n=10)	ND (n=5)
OP	ND ~0.09 (n=10)	ND ~0.6 (n=5)	ND (n=10)	ND (n=5)

ND : not detected

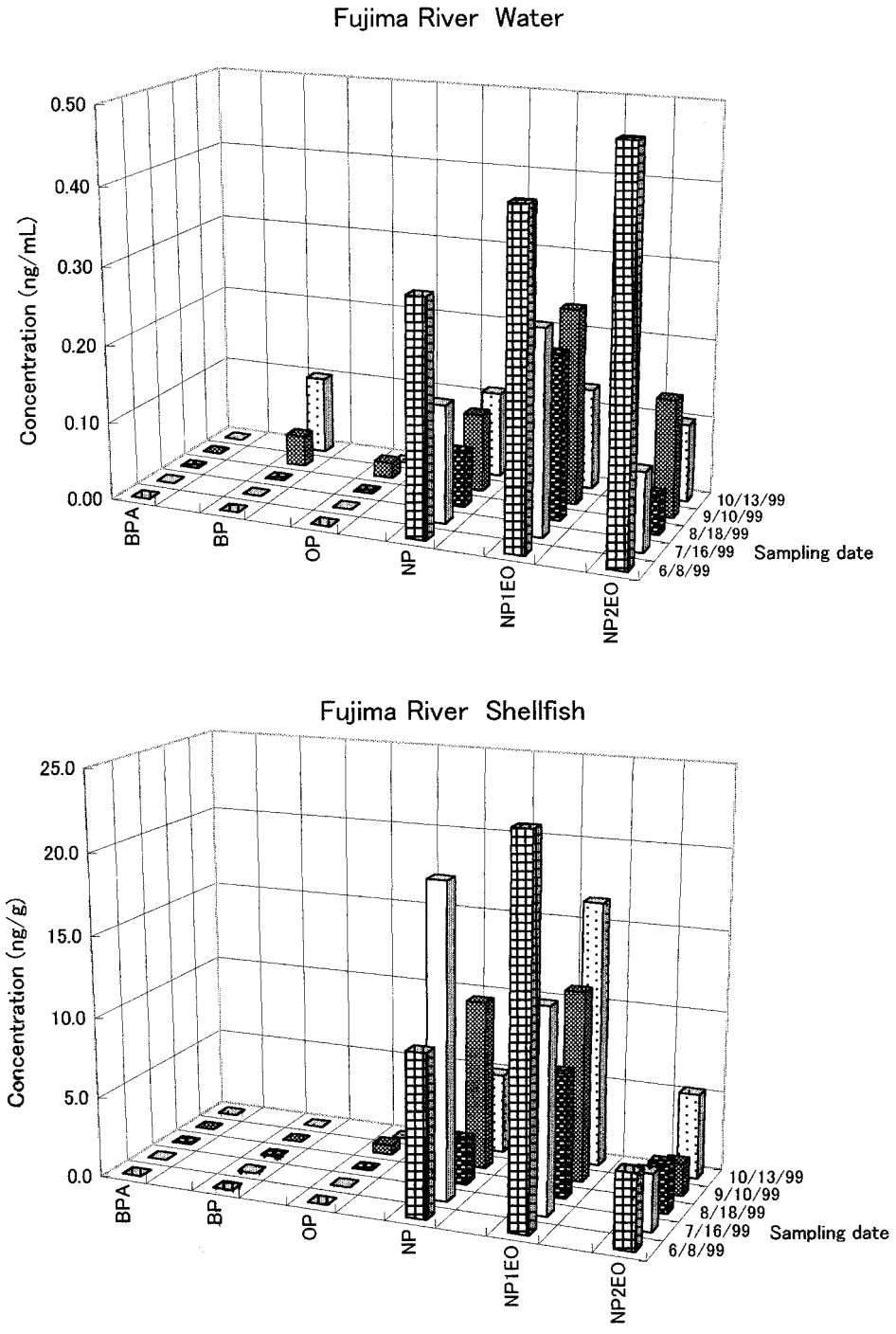

Figure 1. Concentration changes of NP, NP1EO, NP2EO, BPA, BP and OP in water and snails obtained from the Fujima River from June to October in 1999.

An example of the concentration changes of NP, NP1EO, NP2EO, BPA, BP and OP in the water and shellfish from Fujima River is shown in Figure 1 throughout the survey from June to October 1999. Detection of NP, NP1EO and NP2EO in the shellfish corresponded well to that in the water. No detection of BP in the shellfish, in spite of its detection in the water, is probably due to its low bioconcentration potential.

Table 2. Comparison of the present field BCF data with other field BCF data

	The present BCF data (wet weight)		Other BCF data (wet weight)
Chemicals	River snail	Melanian snail	Mussel[a]
NP	70±37 (n=5)	66±27 (n=5)	340
NP1EO	63±26 (n=5)	19± 4 (n=5)	170
NP2EO	34±18 (n=5)	53±21 (n=4)	100

[a] Data from Wahlberg et al. (1990)

Average BCF values of NP, NP1EO and NP2EO in river snail and melanian snail were calculated from the field data (Table 1) and are summarized in Table 2 as field BCF data together with other field BCF data (Wahlberg et al. 1990). The order of BCF in the River snail was equal to that in the Mussel (NP > NP1EO > NP2EO). This order is presumed to be reasonable based on the polarity of the chemicals (NP < NP1EO < NP2EO). For Melanian snail, the order (NP > NP2EO > NP1EO) was different from that of the River snail and the Mussel. The reason for the difference is not obvious from the data of the present study.

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