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1,3,5-Trichloro-2-(4-nitrophenoxy)benzene (CNP) in Water, Sediments, and Shellfish of the Ishikari River

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1,3,5-Trichloro-2-(4-nitrophenoxy)benzene (CNP) is used in large quantities as a paddy herbicide in Japan, and unusually high concentrations of CNP were reported to exist in river water, freshwater fish and shellfish in Honshu districts during the rice planting season (Kanazawa and Tomizawa 1978, Watanabe *et al.* 1981, Ishikawa *et al.* 1981). In Hokkaido, it was also reported that CNP was found in water and a freshwater fish of the Ishikari River (Kaneshima *et al.* 1984).

Since organochlorine compounds are known to be accumulated in benthic animals and CNP is very persistent in aquatic animals (Yamagishi *et al.* 1978), shellfish might be useful as an indicator of environmental contamination by CNP. In order to understand the correlation between the concentrations in shellfish, water and sediments, it is necessary to investigate their temporal changes and the residue half-life time of CNP in the environment. For this purpose, CNP-free shellfish (*Corbicula japonica*) was transfered to fixed point in the lower reaches of the Ishikari River, and the CNP concentrations in shellfish, water and sediments, before and after CNP application, were examined biweekly from May to August and monthly from September to December 1984. The present paper will show that shellfish can be a biological indicator for CNP contamination in the river.

MATERIALS AND METHODS

A total of 37 samples of river water, river-bottom sediments and shellfish (*C. japonica*) were collected at a fixed sampling point in the lower reaches of the Ishikari River (Figure 1). About 500 kg of the CNP-free shellfish harvested in the Teshio River (Figure 1) was placed in the Ishikari River.

The mean weight of the shellfish was 10.1 ± 1.5 g, and their ages were determined to be 6-8 years by means of

Figure 1. Location of sampling points in Hokkaido

the shell-reading method. Shucked shellfish (20 g) were placed in 150 ml of acetonitrile and homogenized with a Polytron tissue homogenizer. And then filtrate was shaken twice with each 100 ml of n-hexane. The combined n-hexane solutions were concentrated to 5 ml. For cleanup, the solution was chromatographed on a silver nitrate-Florisil column followed by a Florisil column without silver nitrate as previously reported by Suzuki *et al*. (1979).

River-bottom sediments (20 g dry weight) were shaken with 150 ml of acetone for 30 min, and the mixture was filtered. The filtrate was shaken twice with each 100 ml of n-hexane. River water (500 ml) was shaken directly twice with each 150 ml of n-hexane. The each n-hexane solution from sediments and water were concentrated to 5 ml, and chromatographed for cleanup in the same manner as described above.

Measurement of CNP was performed with a Shimadzu GC-4BM equipped with a ^{63}Ni electron-capture detector and a 2 m × 4 mm i.d. glass column packed with 2% DEGS + 0.5% phosphoric acid on Chromosorb W.

RESULTS AND DISCUSSION

Figure 2 shows the results of periodic measurements of CNP concentrations in water and sediments collected at a fixed sampling point. CNP in water was found to reach a maximum level of 0.42 ppb on May 29 and was no longer detected (limit of detection was 0.01 ppb) after July 10. It was found that concentration of CNP in

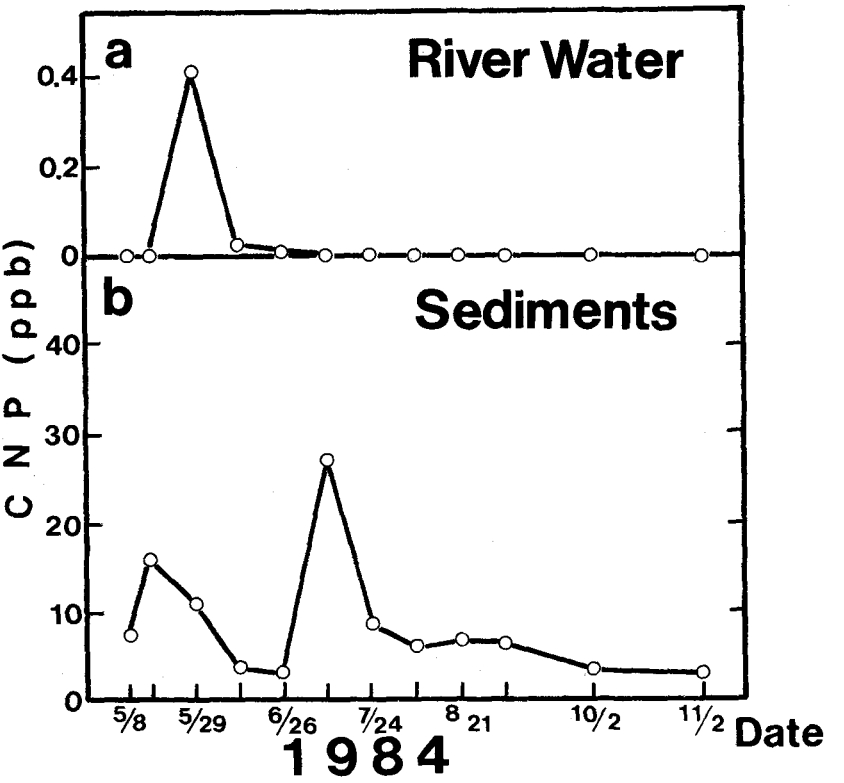

Figure 2. Change of CNP concentrations in water (a) and sediments (b) from the Ishikari River

river water in the Ishikari basin reached the maximum approximately one week after the start of CNP application in rice paddy fields. The distance between the centers of paddy fields and sampling point is about 150 Km. Yamagishi and Akiyama (1981) reported that the CNP concentrations in sea water at the mouths of several rivers discharged into Tokyo Bay ranged from 0.02 to 1.9 ppb during May and July. Suzuki *et al.* (1978) also reported that the CNP concentrations in river water in North Kyushu, Japan, ranged from 0.09 to 17 ppb during June and September. Thus, the periods of the maximum CNP level in river water or sea water, so far reported, are concentrated in June, a CNP application period.

The CNP concentrations found in river-bottom sediments ranged 2.7 to 27 ppb through the sample-collecting period (Fig. 2b). The maximum level was detected in the sample collected on July 10, though the maximum CNP concentration in river water was obtained on May 29.

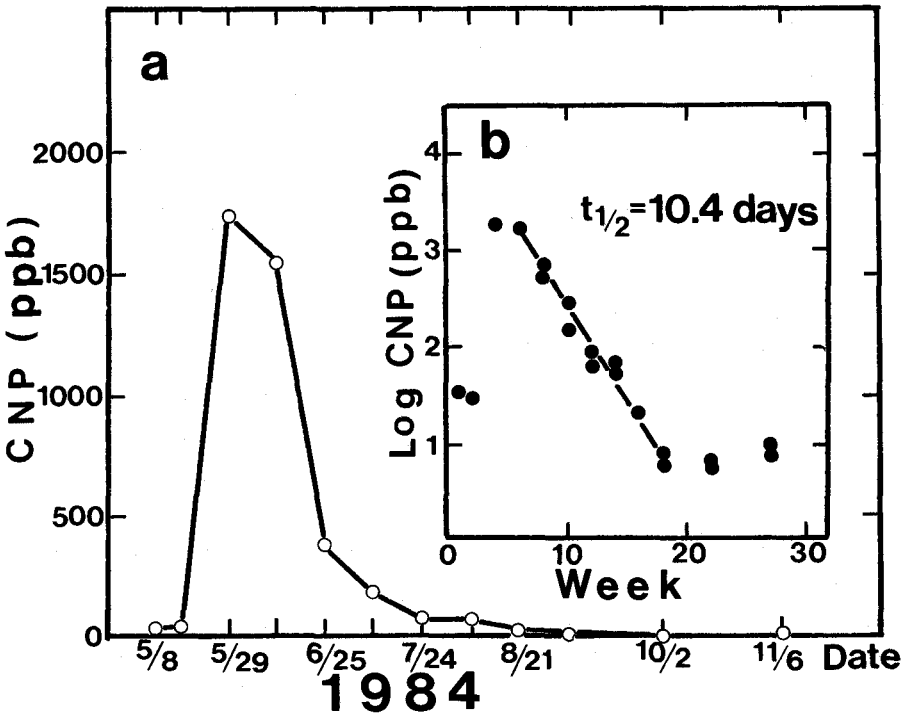

Figure 3. Change of CNP concentrations in shellfish (*C. japonica*) which were periodically sampled at the fixed point at the Ishikari River (a) and logarithm of the CNP concentration in the shellfish against time (b)

The fluctuation in the CNP concentration in sediments might be attributable to the inhomogenity of the sediments arised inevitably when they were collected.

Figure 3 shows results of periodic measurements of the CNP concentration in the shellfish at the fixed sampling point. The maximum CNP concentration (1.7 ppm) was found on May 29 in the shellfish as well as in river water, and the bioconcentration ratio (concentration in a biological material/concentration in water) of CNP was approximately 4,000 for the shellfish. Watanabe *et al.* (1983) reported that the bioconcentration ratio of CNP in freshwater fish was supposed to be 420-8,000. On the other hand, Kobayashi (1977) reported a value of 2,000 for the ratio in fish.

During the decreasing period of the CNP concentration in the shellfish, a linear relationship was observed between the logarithm of the CNP concentration and the elapsed time as shown in Fig. 3b. The half-life time

of CNP in the shellfish was calculated to be 10.4 days from the slope of the curve. But the log-linear relationship was no longer applicable 20 weeks after CNP application. This suggests that the residue of CNP in the shellfish might be obtained from the sediments which still hold 3-8 ppb CNP. Yamagishi *et al.* (1981) reported that the CNP concentration in shellfish (*Tapes philippinarium*) decreased with a half-life time of 24 ± 8 days at several sampling stations in Tokyo Bay. This result suggests that CNP in Tokyo Bay was continuously supplied from agricultural areas in the river basins discharged into Tokyo Bay. Watanabe *et al.* (1985) also reported that the biological half-life time of CNP in bivalve mussels (*Mytilus edulis*) was 4-8 days under laboratory experiments. This discrepancy might be attributable to the difference in the species of the shellfish or in the extent and period of CNP contamination in the environment.

Generally, benthic animals such as shellfish and shrimp accumulate organochlorine compounds like PCB's or DDT. The shellfish used for this work was transfered from the Teshio River to the Ishikari River. The Teshio River was free from CNP and in fact, no CNP was detected in the shellfish in the Teshio River. And the shellfish had disappeared for the past 15 years in the Ishikari River. Therefore, all CNP found in this shellfish was concluded to come only from the Ishikari River system. This shellfish can be said to be a good indicator of environmental contamination by CNP from the following reasons; (1) The bioconcentration ratio of CNP was approximately 4,000 for the shellfish; and (2) the disappearence of CNP in the shellfish followed a first order decay with a definite half-life time. On the other hand, the river water and the sediments as an indicator have following problems; (1) The concentration in river water was much lower than that in the shellfish and was detected only during a short period, June; and (2) the CNP concentration in sediments exhibited a large fluctuation attributed to the inhomogenity of sediment samples.

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