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Fate and Behavior of Herbicides, Butachlor, CNP, Chlomethoxynil, and Simetryne in River Water, Shellfish, and Sediments of the Ishikari River

Tohru Ohyama, Kazuo Jin, Yoshinobu Katoh, Yoshiaki Chiba, and Katsuhiro Inoue

Hokkaido Institute of Public Health, N19, W12, Kita-Ku, Sapporo 060, Japan

It has been noticed that herbicides applied on paddy fields flow out with effluent water to cause contamination of river water and benthic animals in the river. Nowadays, in Hokkaido, herbicides such as butachlor [2-chloro-2', 6' -diethyl -N- (buthoxymethyl) acetanilide], CNP [1,3,5-trichloro-2-(4-nitrophenoxy) benzene], chlomethoxynil [2,4-dichloro-1-(3-methoxy-4-nitrophenoxy)benzene] and simetryne[2,4-bis (ethylamino) -6-methylthio-1,3,5-triazine] are applied extensively on paddy fields, though their applications are restricted during the rice planting season, May to June. However, few studies have been carried out on the contamination of herbicides, other than CNP(Yamagishi *et al.* 1981, Kaneshima *et al.* 1984, Ohyama *et al.* 1986) in aquqtic environments.

The bulk of herbicides applied are adsorbed and retained on the soil during irrigation (Watanabe *et al.* 1984, Chen and Chen 1979). But the concentrations of herbicides washed into the rivers by draining or rain falls could be of the order of ppb in river water. In order to understand accurately the behavior of the herbicides in the aquatic environment of the Ishikari River, it is necessary to obtain a good biological indicator having high bioconcentration ratio. Recently, we reported that shellfish (*Corbicula japonica*) can be a good indicator of environmental contamination by CNP (Ohyama *et al.* 1986). Therefore, the concentrations of four herbicides (butachlor, CNP, chlomethoxynil and simetryne) in the shellfish, river water and sediments, before and after herbicides application, were examined biweekly from May to August and monthly from September to December 1986. The present paper will show that the examination of the shellfish used as the indicator species elucidates the amount and range of herbicides contamination in the aquatic environment of the Ishikari River system, and that bioconcentration and biological half-life time of the herbicides for the shellfish (*C. japonica*) correlate with water solubility of the herbicides examined.

MATERIALS AND METHODS

A total of 33 samples of river water, river bottom sediments and shellfish (*C. japonica*) were collected at the fixed sampling point in the lower reaches of the Ishikari River.

The mean weight of the shellfish was 10.1 ± 1.5g, and their ages were determined to be 7-9 years by means of the shell-reading method. Shucked shellfish (20 g) were placed in 150ml of acetonitrile and homogenized with a polytron tissue homogenizer. The filtrate was shaken twice with each 100ml of dichloromethane. The dichloromethane solutions were combined, evaporated and then dissolved in 5ml of n-hexane.

River bottom sediments (20 g dry weight) were shaken with 150ml of acetone for 30 min, and the mixture was filtered. The filtrate was shaken twice each 100ml of dichloromethane. The each dichloromethane solution from sediments and water was evaporated and dissolved in 5ml of n-hexane.

For cleanup, the each n-hexane solution from shellfish, sediments and water were chromatographed on a Florisil column and silver nitrate-Florisil column as previously reported by Suzuki *et al.* (1979). Butachlor, CNP and chlomethoxynil were recovered by elution with 150ml of a mixture of ether-hexane (50:50), and then simetryne was recovered by elution with 150ml of a mixture of acetone-hexane (15:85). Each fraction was evaporated to 0.5ml for water samples, 5ml for sediments and 10 ml for the shellfish. The concentrates were analyzed on two types of gas chromatographs, a Shimadzu 4BM equipped with a electron capture (^{63}Ni) detector and a Shimadzu 7A equipped with a flame thermionic detector.

Gas chromatography was performed with two packed glass columns: column 1, 2% DEGS + 0.5% phosphoric acid on Chromosorb W; column 2, 5% Silicon DC-200.

RESULTS AND DISCUSSION

Figure 1 shows the results of periodic measurements of herbicides concentration in water and shellfish collected at a fixed sampling point in the lower reaches of the Ishikari River. Butachlor, which is mostly applied on paddy fields in Hokkaido, in water was found to reach a maximum level of 4.4 ppb on May 21. The maximum levels of CNP and chlomethoxynil were

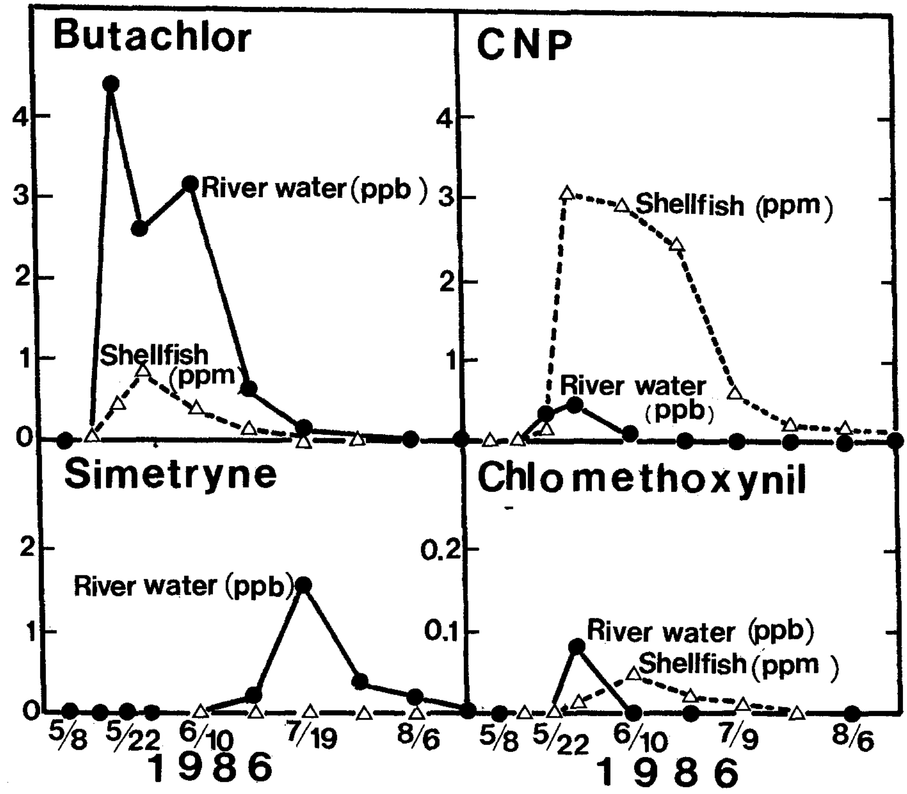

Figure 1. Changes of herbicides (CNP, butachlor, simetryne and chlomethoxynil) concentrations in water and shellfish (*C. japonica*) which were periodically collected at the fixed point at the Ishikari River. (●——●)River water and (△·····△)shellfish.

latened for 1 week on May 28. On the other hand, the maximum level of simetryne was detected in the River water on July 1st, approximately one month after the maximum levels of other herbicides examined.

Usually, the application period of these herbicides, which is applied just before and after rice planting period, is in the middle of May, except for simetryne. It was found that the concentration of herbicides in river water in the Ishikari basin reached the maximum approximately one week after the start of first application in rice fields. The distance between the centers of paddy fields and sampling point is about 150 km.

Simetryne is normally applied on the field 3 weeks after rice planting. The period of time for reaching the maximum level of each herbicide in river water was consistent with the period the one week after the bioconcentration factor in rainbow trout and the aqueous solubilities for some stable organic compounds.

During the decreasing period of CNP, butachlor and chlomethoxynil concentrations in the shellfish, a linear relationship was observed between the logarithm of the concentration of each herbicide and the elapsed time as shown in Figure 2a. The half-life times of the herbicides examined in the shellfish were calculated to be 10.4 days for CNP, 9.1 days for chlomethoxynil and 3.7 days for butachlor from the slopes of the curves.

Although the maximum concentrations of CNP in the shellfish from 1984 to 1986 were slightly different from those in water, the biological half-life time of CNP for the shellfish was always 10.4 days in the past three years (unpublished data). This indicates that the annual spraying of the herbicides was always carried out during quite short period. Generally, the half-life time of CNP depends upon the length of the herbicides application period. Yamagishi *et al*. (1981) reported that the half-life time ranged from 24 to 34 days for the shellfish (*Tapes philippinarium*) collected at several sampling stations in Tokyo Bay. This is simply attributable to the fact that CNP at the mouth of the rivers discharged into Tokyo Bay was continuously supplied from agricultural areas in the river basin. On the other hand, under laboratory experiments, half-life time was 4-8 days for bivalve mussel (Watanabe *et al*. 1985).

With respect to butachlor, chlomethoxynil and simetryne, there have been few definite data about biological half-life time for benthic animals in aquatic environments. By only one report on chlomethoxynil, it was shown to be 1.6 days for mussel under laboratory experiments after being exposed to CNP for 9 days (Watanabe *et al*. 1985).

We attempted to correlate values of half-life time of herbicides with water solubility of herbicides, resulting that there was a linear relationship between them, as shown in Figure 2b. This indicates that the rate of disapperence of herbicides in the shellfish simply depended upon the water solubility as was in the case of bioconcentration ratio. The behavior and fate of the herbicides or other chemicals in the aquatic environment of the Ishikari River might be able to estimated by the determination of the water solubility of the chemicals. Therefore, these correlations among water solubility, biological half-life time and bioconcentration ratio will be useful in assessing the possible contamination and in considering the effects of the chemicals on the environment.

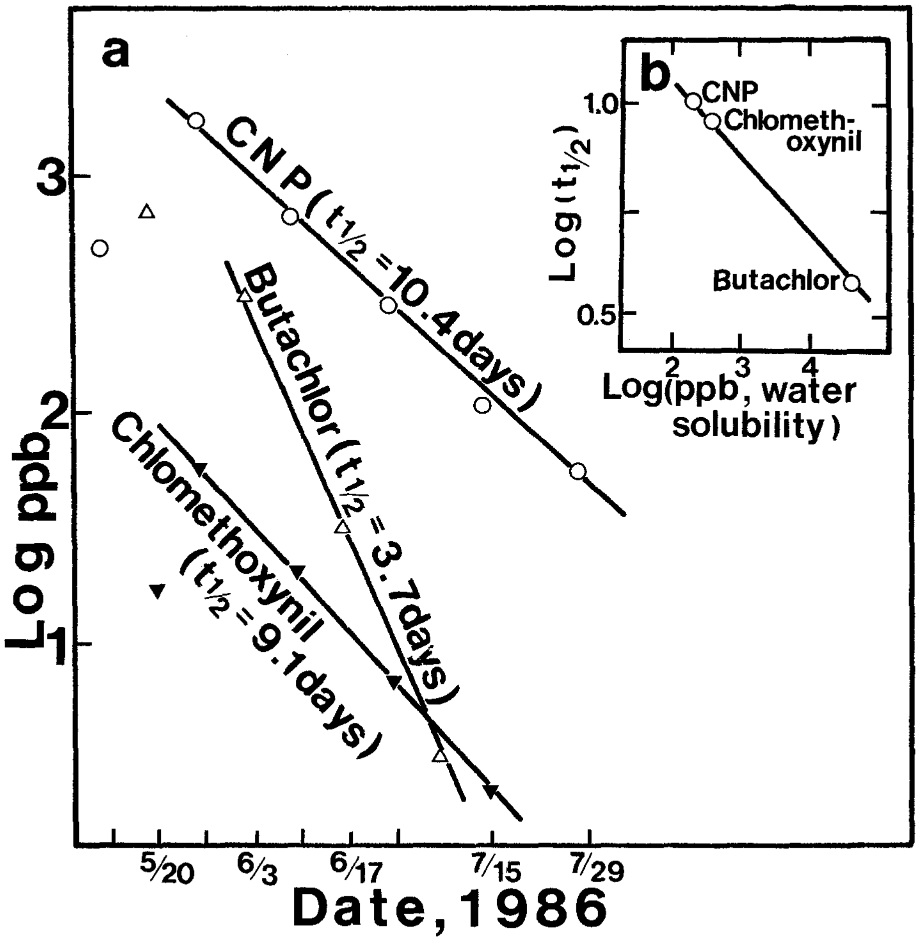

Figure 2. Logarithm of the herbicides concentrations in the shellfish against time (a) and the relationship between the water solubility and the biological half-life time of herbicides in the shellfish of the Ishikari River (b).

bioconcentration ratio obtained from *C. japonica* followed the above equation for CNP, butachlor and chlomethoxynil. Therefore, it might be said that *C. japonica* and *M. edulis* behave in a similar manner in view of the bioconcentration of the herbicides. When water solubility of simetryne (450 ppm, 25°C) applies to the above equation, the bioconcentration ratio of 16.6 can be available. Then, as the concentration of simetryne found in river water was 1.5 ppb at maximum level, the concentration of simetryne in the shellfish must be 25 ppb at maximum level which was value around the detection limit (10 ppb). In fact, simetryne was no longer detected in the shellfish within the detection limit of 10 ppb. On the other hand, Chiou *et al.* (1977) also obtained a similar equation between the application of each herbicide. In Honshu districts, the periods of maximum herbicides level in river water, so far reported (Yamagishi and Akiyama 1981, Watanabe *et al*. 1983), are also concentrated in June, herbicides application period. All herbicides applied during rice planting season were no longer detected after the end of July within the detection limits of 0.01 ppb.

Annually, the proportions of the total herbicides applied on agricultural area of the Ishikari basin are 60% of butachlor, 10% of CNP, and a few % of simetryne and chlomethoxynil. The maximum level of each herbicide except simetryne in the river water may reflect the amount of individual herbicides applied on agricultural area of the Ishikari basin. Watanabe *et al*. (1984) suggested that the amount which flow out from the paddy field with water was 22-26% in simetryne and 1.3-1.6% in CNP. This might be a plausible explanation for the results that the concentration of simetryne in water of the Ishikari River was higher than CNP in spite of its small amount of application.

Figure 1 also shows the results of periodic measurements of the herbicides concentrations in the shellfish (*C. japonica*) at the fixed sampling point at the same time as sampling water. The maximum concentrations of the herbicides except simetryne were observed at the end of May. The maximum levels of CNP, butachlor and chlomethoxynil were 3.0, 0.17 and 0.06 ppm, respectively. Simetryne was not detected in any of the shellfish examined (limit of detection was 10 ppb). These results did not coincide with those obtained from water samples. Then, the bioconcentration ratios (concentration in a biological material/concentration in water) of herbicides were calculated. As a result, the values obtained were 2,000 for CNP, 500 for chlomethoxynil and 250 for butachlor.

With respect to the correlation of bioconcentration with physicochemical properties of herbicides, Watanabe *et al*. (1985) reported that an inverse correlation was observed between the bioconcentration ratio for bivalve mussel (*Mytilus edulis*) and the water solubility of the four herbicides, molinate, benthiocarb, CNP and chlomethoxynil, under laboratory experiments. They yielded the following equation with a correlation coefficient of -0.96.

$$\mathrm{Log}[C] = -0.58\,\mathrm{Log}[S] + 4.5$$

For this equation, where [C] was bioconcentration ratio for mussel and [S] was the water solubility (ppb) of the herbicides examined. Our results of

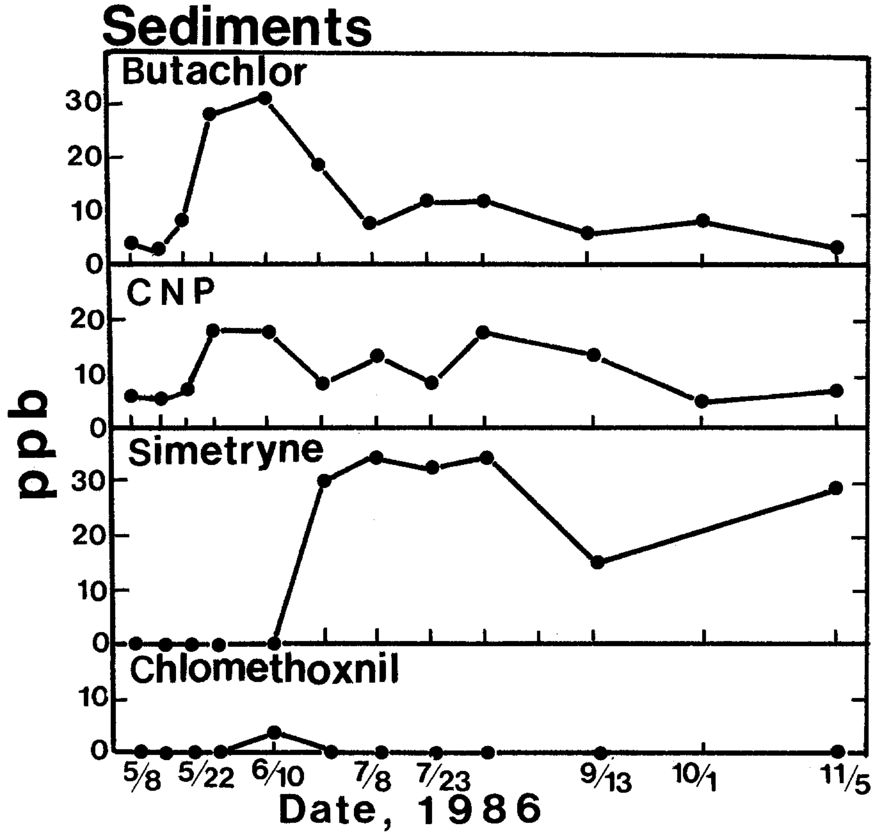

Figure 3. Changes of herbicides (butachlor, CNP, simetryne and chlomethoxynil) concentrations in river-bottom sediments from the Ishikari River,1986.

The herbicides concentrations found in river- bottom sediments ranged from N.D. to 50 ppb through the sampling period (Figure 3). The dates on the maximum level of each herbicide were all different with the herbicides examined. It appeared that there was no distinct correlation between the concentration of the herbicides in sediments and the water solubilities of the compounds, as different from the case of shellfish. The fluctuation in each herbicide concentration in sediments might be attributable to the inhomogeneity of the sediments arised inevitably when they were collected. Although the sediments as an indicator have such problem, sediments are still important materials to elucidate the behavior and fate of the herbicides in aquatic environments by the fact that the sediments hold herbicides for long term.

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