

Seasonal and Spatial Studies on Pesticide Residues in Surface Waters of the Shinano River in Japan

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Water samples collected once a week from early April 1996 to the end of August 1996 from four sites on the Shinano River in Japan were analyzed for pesticides and their metabolites using a gas chromatograph–mass spectrometer. Among the total of 53 chemicals found, 22 were herbicides, 15 were insecticides, 11 were fungicides, and 5 were metabolites. The concentrations of chemicals found ranged from 3 ng/L (bromobutide) to 8200 ng/L (isoprothiolane). Herbicides were found primarily during May and June at all four sites. Insecticides and fungicides were found primarily during July and August at all four sites. The presence of pesticides in the river water correlated with the time of pesticide application to the rice fields near the river.

Keywords: GC-MS; pesticides; river water; Shinano River

INTRODUCTION

Even though governments of various countries set acceptable residue levels for specific pesticides, public concern still focuses on the fate of pesticides in a food chain. Rivers are one of the most important water sources for agriculture as well as for drinking water. Therefore, pesticide contamination of river water has received much attention from environmental chemists. Many studies have monitored pesticide residues in major rivers. In particular, the fates of pesticides in the Mississippi River and its tributaries have been investigated intensively because farmlands in its basin are major recipients of herbicides applied to corn, soybeans, sorghum, rice, and cotton. For example, 23 compounds, including organophosphorus pesticides, were identified in the water collected from nonpoint sources of the Mississippi River (1). There are many reports of spatial and seasonal variability studies on the fates of pesticides in the Mississippi River because it is an extremely large and long river (2–4).

The Shinano River is the largest river in Japan. It flows 366.6 km from Kobushin Peak at the border of the Nagano, Saitama, and Yamanashi prefectures to the Sea of Japan at the city of Niigata. It is approximately $1/10$ the length of the Mississippi River. The Shinano River basin is one of the largest watersheds in Japan and drains an area of ~ 11900 km² in four prefectures. For several centuries, there have been rice fields along the Shinano River. In addition to being used for the generation of electricity, Shinano River water is used for agriculture (80.5%), drinking water (3%), industry (14.4%), and other uses (2.1%).

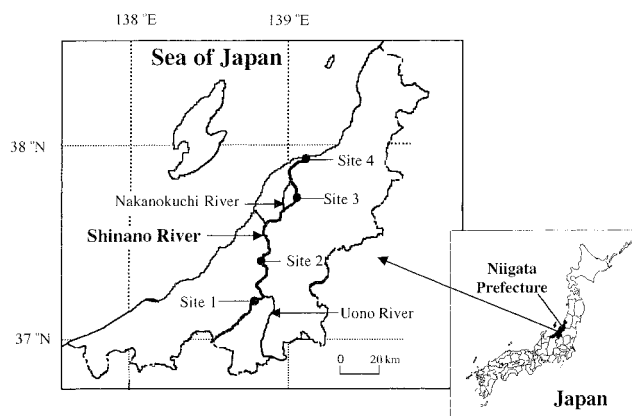


Figure 1. Location of the Shinano River in Japan and the sampling sites.

Over 300 pesticides have currently been registered in Japan. In Niigata prefecture, a typical rice-growing region, many pesticides have been used in the rice fields. Consequently, river water may contain many pesticides and their metabolites. It is necessary to analyze pesticides and their metabolites in river waters to assess their fate in the environment. In the present study, pesticides and their metabolites in water collected from various sites on the Shinano River were analyzed to investigate seasonal and spatial variations of pesticide residue levels.

EXPERIMENTAL PROCEDURES

Chemicals. Standard pesticides and their metabolites were purchased from Wako Pure Chemical Industries (Osaka, Japan) and GL Science (Tokyo, Japan). Polyethylene glycol (PEG 200 and PEG 300) were bought from Wako Pure Chemical Industries. Each standard pesticide was dissolved

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Table 1. Amounts (Nanograms per Liter) of Pesticides Found in Waters Collected from the Shinano River, Japan

	LOD ^a	LOQ ^b	site 1			site 2			site 3			site 4		
			range	av	n ^c	range	av	n	range	av	n	range	av	n
Herbicides														
alachlor	4	10	14–14	14	4			0	20–22	21	8	18–18	18	4
ametryn	5	14			0	16–54	35	8	18–18	18	4	20–20	20	4
atrazine	4	10	110–170	140	8	98–150	130	13	60–150	120	17	28–150	60	67
bromobutide	2	6	3–240	67	63	4–440	130	67	6–240	88	71	5–610	150	83
butachlor	3	12	20–28	23	21	20–130	52	25	18–32	27	21	22–30	27	25
butamifos	5	13			0	34–34	34	4	26–32	29	8	18–28	23	8
dichlobenil	3	8	6–32	19	33	10–18	15	29	4–54	24	42	12–76	29	96
dimetipiperate	4	12	14–750	210	25	48–5800	1300	38	16–250	92	29	12–560	200	38
dimethametryn	3	8	14–14	14	4	10–120	76	21	12–16	14	17	10–30	20	29
esprocarb	5	13	36–100	60	29	20–340	140	33	38–210	110	38	10–470	96	67
linuron	5	14	58–90	74	8	14–48	31	8	14–14	14	4	10–28	18	25
mefenacet	4	11	26–2200	470	38	14–3200	830	54	12–1000	280	58	16–1700	410	54
molinate	3	9	10–160	75	25	6–220	84	38	8–740	170	71	8–990	190	75
oxadiazon	3	9	13–16	15	8	18–20	19	8	12–12	12	4	10–22	19	21
piperophos	7	18			0	24–350	240	17	30–42	36	8	22–28	25	13
pretilachlor	3	9	16–490	160	46	17–5200	710	54	14–1100	310	50	16–1600	460	46
prometryn	4	11	12–12	12	4			0			0			0
pyributicarb	3	9	14–82	42	21	14–700	240	29	40–220	90	29	22–430	160	29
simazine	4	11	14–180	99	8	24–24	24	4	18–18	18	4	16–16	16	4
simetryn	3	8	10–230	57	58	12–1600	170	79	14–310	92	71	10–330	82	79
thenylchlor	4	10	30–50	37	13	16–120	59	25	32–110	68	17	20–320	110	25
thiobencarb	3	8	12–320	76	50	18–4300	700	50	10–210	88	54	14–200	83	50
Fungicides														
edifenphos	5	13			0	12–12	12	4	10–10	10	4	16–16	16	4
etridiazol	5	12	18–18	18	4			0			0			0
flutolanil	2	6	12–68	31	17	16–28	21	25	12–200	58	29	12–52	32	33
fthalide	4	10	14–130	49	42	8–990	120	54	14–440	140	38	8–640	150	71
iprobenfos	4	12	12–870	180	33	12–230	88	50	12–260	81	42	12–210	79	50
isoprothiolane	5	12	20–8200	810	75	12–1300	290	67	16–2600	430	71	24–1500	360	58
mepronil	3	9	14–870	250	17	10–190	63	17	14–850	230	25	16–900	310	29
pencycuron	7	18	28–28	28	4	300–300	300	4			0	20–60	40	13
probenazole	8	21	46–300	140	13	28–200	93	13	270–270	270	4	46–160	92	13
quintozene	7	20	24–35	30	8	22–26	24	8	20–20	20	4	14–24	19	8
tricyclazole	30	81	34–36	35	8	52–7700	2900	13			0	138–580	350	29
Insecticides														
buprofezin	5	12	12–12	12	4	12–12	12	4	14–14	14	4	12–28	20	13
carbaryl	2	6			0	36–590	310	8			0	10–74	29	17
chlorfenvinphos	5	13			0			0			0	12–12	12	4
chlorpyrifos	5	14			0	10–10	10	4	7–7	7	4			0
cyanophos	3	9	10–44	22	17	12–18	15	13	12–12	12	4			0
diazinon	4	12	12–140	48	38	14–320	57	42	12–60	31	33	10–50	24	54
dichlofenthion	3	7			0			0			0	10–10	10	4
dichlorvos	3	8			0			0	82–82	82	4	12–26	19	8
dimethoate	9	25	26–26	26	4	200–200	200	4	34–34	34	4			0
etofenprox	3	8			0	4–200	56	17	14–37	26	8			0
fenitrothion	6	17	22–84	36	38	14–1700	210	38	16–330	51	88	16–280	58	79
fenobucarb	3	8	12–56	29	46	16–1300	150	46	12–330	72	50	12–100	43	46
malathion	4	11	23–23	23	4			0			0	28–28	28	4
propxur	3	9			0	11–84	34	29	12–16	14	8	12–180	47	33
pyridaphenthion	7	19	22–22	22	8	20–28	24	8	36–92	56	17	22–140	59	17
Metabolites														
isofenpos oxon	7	20	45–45	45	4	12–39	25	8			0	30–120	79	13
tolchlofos methyl oxon	3	7			0		0	16–16	16	4			0	
2,6-dichlorobenzamide	5	14	16–62	34	33	12–118	31	29	12–110	46	42	12–240	88	92
bromobutide-debromo	3	8	4–27	14	46	4–29	15	46	4–38	17	54	8–56	23	71
butamifos oxon	3	9			0	18–18	18	4	14–14	14	4	10–14	12	13

^a LOD, limit of detection. ^b Limit of quantification. ^c $n = (\text{number of samples containing pesticide}/\text{number of samples analyzed}) \times 100$.

into acetone to make up a 1 mg/mL solution (the simazine solution was 0.1 mg/mL). A stock solution (2 μg of each pesticide/mL) of each pesticide was prepared using a standard pesticide solution. Internal standard solutions (200 $\mu\text{g}/\text{mL}$ of acetone) of 9-bromoanthracene (Aldrich, Milwaukee, WI) and 1,4-diiodobenzene (Tokyo Kasei Kogyo, Tokyo, Japan) were also prepared. A 10 mg/mL acetone solution of PEG was prepared from PEG 200 and PEG 300 (1:1, v/v). The PEG solution was used to increase the peak intensity of certain pesticides, including pencycuron EPN oxon, benzulide, butam-

ifos oxon, and pyrazoxyfen (5, 6). All chemicals were of pesticide analytical grade.

Sample Collections from the River Waters. Water samples were collected once a week from early April 1996 to the end of August 1996 from the four sites shown in Figure 1. Sites 1, 2, 3, and 4 are 108, 78, 32, and 4 km from the mouth of the Shinano River, respectively. The water samples were placed in 1 L glass bottles and stored at 4 °C in the dark. All samples were analyzed within 24 h after collection.

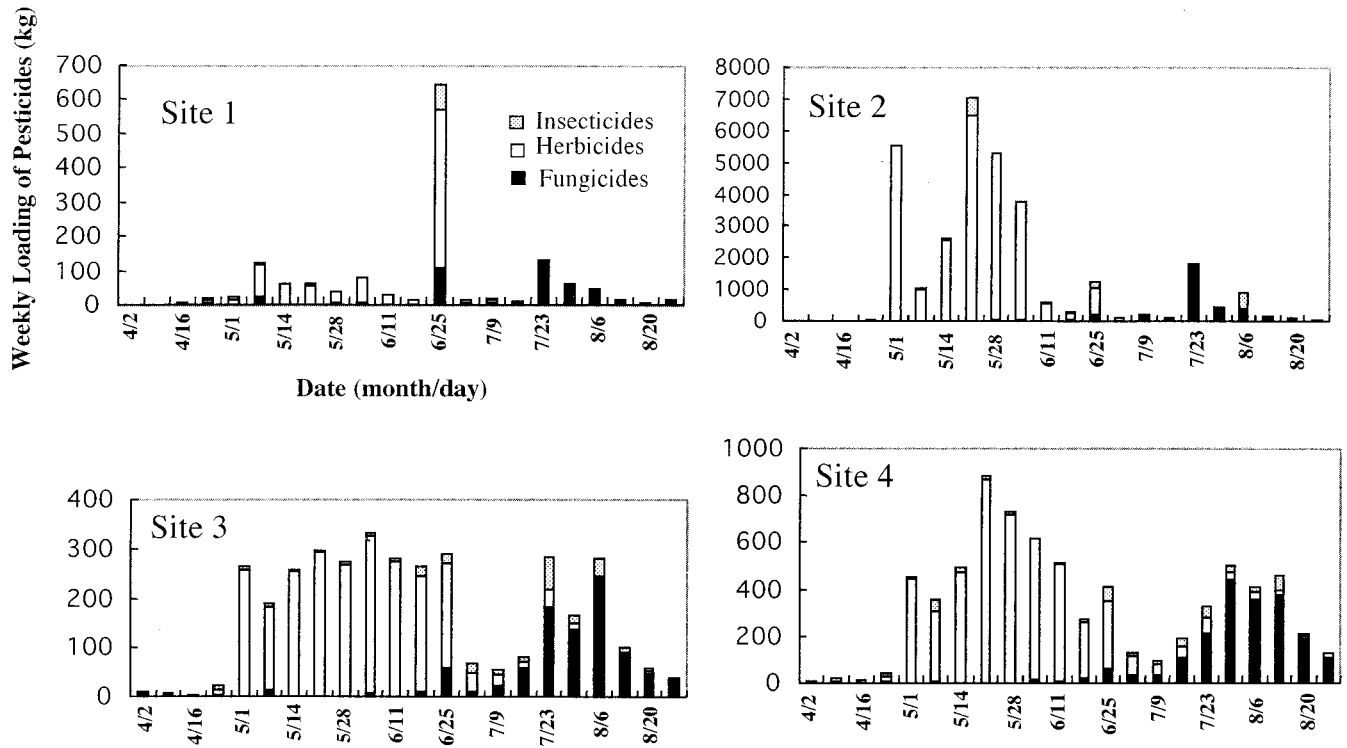


Figure 2. Weekly loading variation of three kinds of pesticides in the river waters at each site.

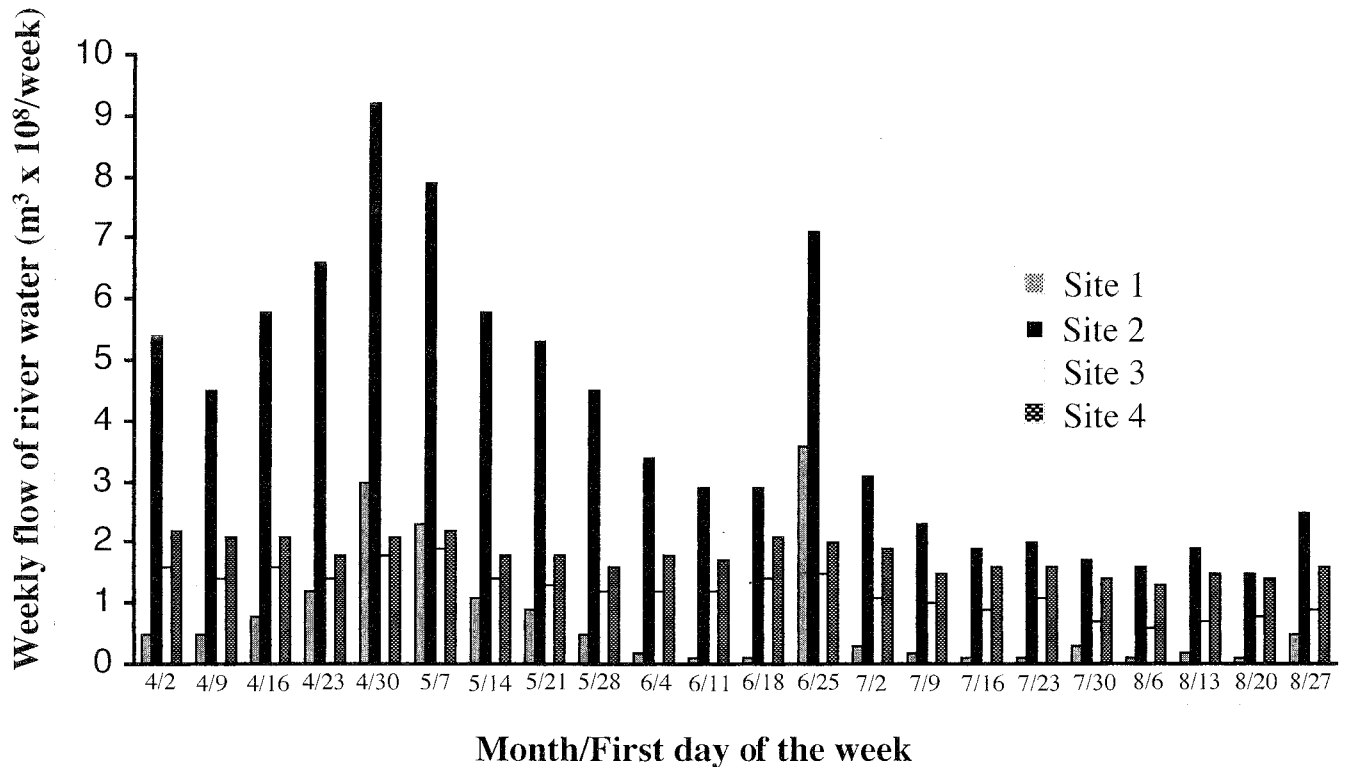


Figure 3. Weekly flow of the river water.

Generally, rice planting is conducted during early May and mid-May in the Shinano River watershed area in Japan. Harvest is done during mid-September and late September. Pesticides are applied throughout from 3 weeks before rice planting and until 1 month before harvest.

Sample Preparations for Analysis of Pesticides and Their Metabolites. Sample waters (500 mL) were filtered

with a 47 mm diameter glass-fiber filter (1 μm pore size, Toyo Roshi GA-100, Tokyo, Japan). The glass-fiber filter was washed with acetone prior to use. The filtrates were passed through a Sep-Pak Plus PS-2 cartridge (styrene divinylbenzene copolymer, 265 mg/cartridge, Nippon Millipore, Tokyo, Japan) at 10 mL/min using a Waters Sep-Pak concentrator (Nippon Millipore). The cartridge was subsequently washed with 10 mL of

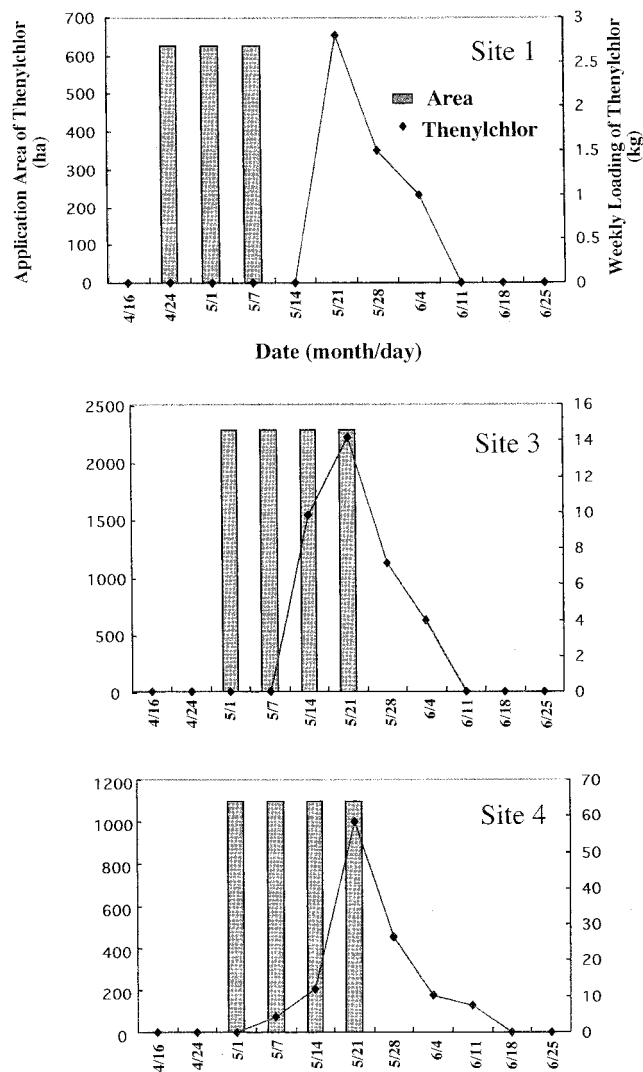


Figure 4. Relationship between weekly loading variations of thenylchlor in the river waters and its application area.

distilled water. The remaining water in the cartridge was removed with an aspirator for 15 min. The organic chemicals trapped on the cartridge were removed with 3 mL of acetone, 3 mL of hexane, and 3 mL of ethyl acetate in series. The eluate from the cartridge was condensed to 1 mL under a purified

nitrogen stream, and then 5 μL of the internal standard solution and 10 μL of the PEG solution were added. The condensed samples were analyzed for pesticides and their metabolites using a gas chromatograph–mass spectrometer (GC-MS).

Solid substances trapped on the glass-fiber filter were extracted with 20 mL of acetone under sonication. The acetone solution was centrifuged at 3000 rpm, and then the upper layer was condensed to 1 mL under a purified nitrogen stream. An internal standard solution (5 μL) and 10 μL of PEG solution were added to the condensed sample. The condensed samples were analyzed for pesticides and their metabolites using a GC-MS.

A standard curve for quantitative analysis was prepared using acetone solutions of each standard pesticide mixture, of which concentrations ranged from 0.01 to 1 $\mu\text{g}/\text{mL}$. A standard curve was prepared for each experiment.

Instrumental Analysis. All samples were analyzed using the Kovats GC retention index I (7) and GC-MS. The GC retention index and MS fragmentation pattern of each component were compared with those of the authentic compound for qualitative analysis. The ratios of peak areas of the selected ions to those of the internal standards (1,4-diidobenzene and 9-bromoanthracene) were used for quantitative analysis of chemicals (8). 1,4-Diidobenzene was used for the chemicals with Kovats index (I) <2000, and 9-bromoanthracene was used for the chemicals with I > 2000.

A Hewlett-Packard 5890 II gas chromatograph interfaced to JEOL model Automass 50 mass spectrometer (JEOL, Tokyo, Japan) was used for qualitative and quantitative analysis of the pesticides and their metabolites. The GC was equipped with a 30 m \times 0.32 mm i.d. ($D_t = 0.25 \mu\text{m}$) HP-5 fused silica capillary column. The oven temperature was held at 50 $^\circ\text{C}$ for 1 min and then programmed to 150 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$ and then to 280 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$. Helium carrier gas pressure was programmed from 2 to 15 psi at 99 psi/min, held for 0.5 min, and then programmed back to 2 psi at the same rate. The injector temperature was 250 $^\circ\text{C}$ with a splitless mode. The GC-MS interface temperature was 230 $^\circ\text{C}$. MS was operated at an ionization current of 350 μA , an ionization energy of 70 eV, a scan mass range of m/z 50–450, and a scan speed of 400 ms/scan. The MS was tuned daily with perfluorotributylamine (PFTBA).

RESULTS AND DISCUSSION

The recovery efficiency study on the target pesticides and related compounds was conducted according to the method developed previously (8). When 16 ng of each of all the standard pesticides except tricy-

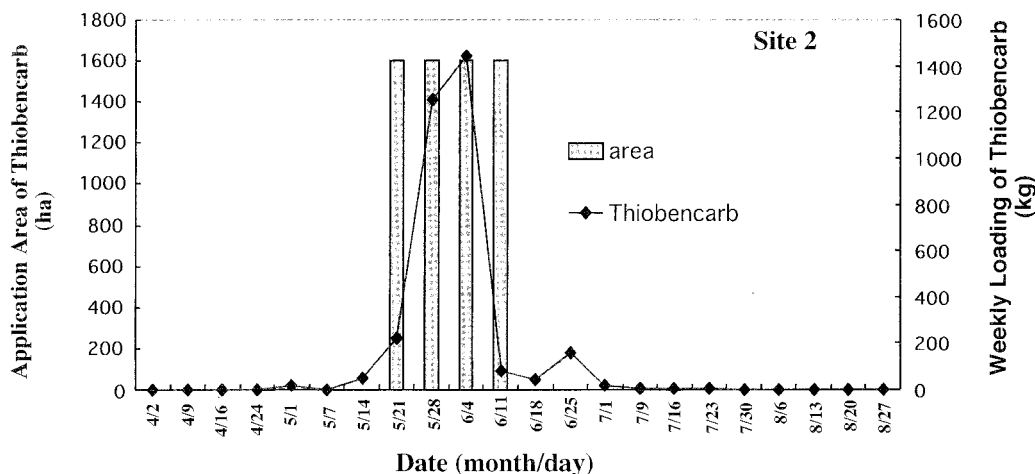


Figure 5. Relationship between weekly loading variations of thiobencarb in the river waters and its application area.

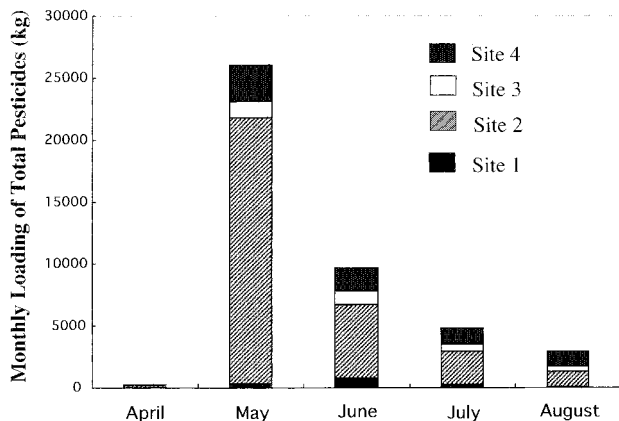


Figure 6. Monthly loading variation of total pesticides in the river waters at four sites.

clazole and their metabolites (22 herbicides, 10 fungicides, 15 insecticides, and 5 metabolites) was added to 500 mL of prefiltered river water, their recovery efficiencies ranged from 83% (etofenprox and tolclofos-methyl) to 107% (isofenphos oxon). The standard deviation ($n = 5$) ranged from 3.9% (flutolanil and carbaryl) to 15.5% (dimethoate). The limits of detection (LOD) ranged from 2 ng/L (bromobutide, flutolanil, and carbaryl) to 9 ng/L (probenazole); the limits of quantitation (LOQ) ranged from 6 ng/L (bromobutide, flutolanil, and carbaryl) to 25 ng/L (dimethoate). When 160 ng/mL of tricyclazole was added to 500 mL of prefiltered river water, its recovery efficiency was $88 \pm 5.7\%$ ($n = 5$). Its LOD and LOQ were 30 and 80 ng/L, respectively.

Table 1 shows pesticides and related compounds found in the river waters collected from the Shinano River. Among the total of 53 chemicals found, 22 were herbicides, 15 were insecticides, 11 were fungicides, and 5 were metabolites. The concentrations of chemicals ranged from 3 ng/L (bromobutide) to 8200 ng/L (isoprothiolane). The levels of pesticides found in the river waters were less than their tolerance levels—which were specified by the Japan Ministry of Environment and/or the Japan Ministry of Health, Labor and Welfare—in a water zone for public use.

Chemicals found in the samples collected at site 1 were 19 herbicides, 8 insecticides, 10 fungicides, and 3 metabolites. Average concentrations of pesticides found ranged from 12 ng/L (prometryn and buprofezin) to 8200 ng/L (isoprothiolane). Among the total of 40 chemicals found at site 1, isoprothiolane was found most frequently (75%) and in the greatest concentration (8200 ng/L). Chemicals found in the samples collected at site 2 were 20 herbicides, 11 insecticides, 10 fungicides, and 4 metabolites. Among the total of 45 chemicals found at site 2, dimepiperate had the greatest concentration (average = 1300 ng/L) and simetryn had the highest frequency value (79%). Chemicals found in the samples collected at site 3 were 21 herbicides, 11 insecticides, 8 fungicides, and 4 metabolites. Among the total of 45 chemicals found at site 3, isoprothiolane had the greatest average value (430 ng/L) and the highest value (2600 ng/L). Fenitrothion had the highest frequency value (88%). Chemicals found in the samples collected at site 4 were 21 herbicides, 11 insecticides, 10 fungicides, and 4 metabolites. Among the total of 46 chemicals found at site 4, pretilachlor had the greatest average concen-

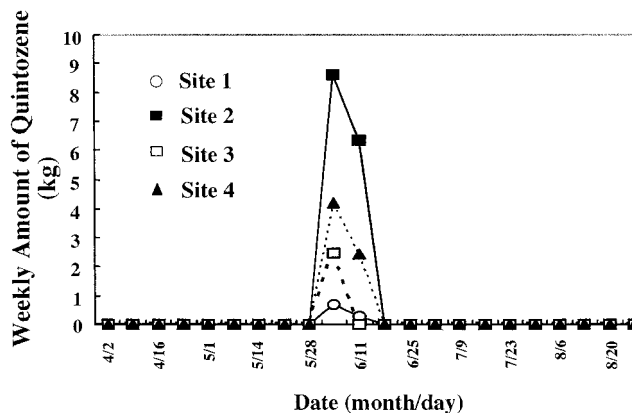
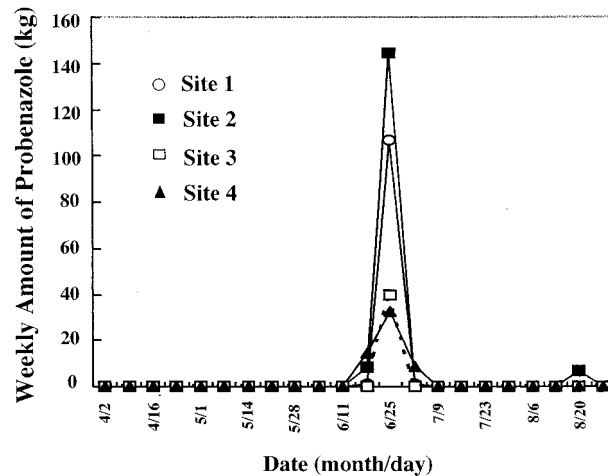


Figure 7. Weekly loading variation of probenazole and quintozene in the river waters.

tration (460 ng/L). Dichlobenil had the highest frequency value (96%).

The results obtained in the present study were similar to those reported in the water collected from seven different rivers that flow into Lake Biwa, Shiga prefecture (9). All of the pesticides found in the present study (except fenthion) have also been found in the river flow into Kasumigaura, Ibaragi prefecture (10). Weekly variations of pesticide loading in the river waters at each site are shown in Figure 2. Figure 3 shows the weekly flow of the river water. The values of river water amounts were measured by the Japan Ministry of Construction Works (11). The water level increased by rainfall in rice fields is adjusted by exhausting excess water into the river through a specific exhaust channel. There is a not specific relationship between rainfall and pesticide loading in river waters.

An approximate weekly pesticide loading was calculated by multiplying the pesticide concentration by the calculated amount of the river water passing through a site in 1 week. Herbicides were found primarily during May and June at all four sites. Insecticides and fungicides were found primarily during July and August at all four sites. The presence of pesticides in the river correlated with the time of pesticide application to the rice fields near the Shinano River. Figure 4 shows the relationship between weekly variations of thenylchlor in the river water and its application area. The application area of site 2 was not available. The results indicated that the pesticide residues drained into the river 2–3 weeks after application. The pesticide

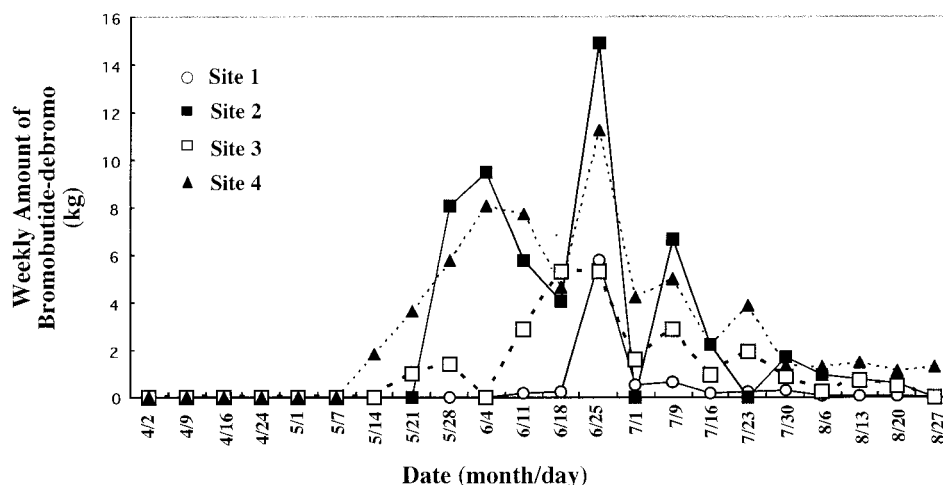


Figure 8. Weekly loading variation of bromobutide-debromo in the river waters.

loading level reduced gradually over 3 weeks after it reached the maximum level. Figure 5 shows the relationship between weekly variations of thiobencarb in the river water from site 2 and its application area. Thiobencarb was applied near site 2 in large amounts, and it was detected immediately after application. The results are not shown here, but the fungicides dimepiperate and pretilachlor showed the same behavior as thiobencarb.

Figure 6 shows the monthly variation of total pesticide loading at the four sites. The total pesticide loading at all four sites was the highest in May when most applications were conducted; it reduced over the following months. Loading was the lowest at site 1, which is the most upstream of the four sites. Loading was highest at site 2 where the Uono River joins the Shinano River; and the loading value was reduced slightly at site 3 where the Nakanoguchi River branched out. Finally, the loading value increased slightly at site 4 where the Nakanokuchi River joins the Shinano River.

Most insecticides were found in the greatest amounts in the waters from all four sites collected from the end of May to the beginning of July. The typical cases of probenazole and quintozone are shown in Figure 7. The results suggest that insecticides were applied around the same time and drained into the river. On the other hand, cases of pesticide metabolites, such as bromobutide-debromo, showed a complex pattern of recovery over time. The typical case of a metabolite, bromobutide-debromo, is shown in Figure 8. Generally, metabolites were found mostly in June, suggesting that degradation occurred in 1 month.

The results obtained in the present study show that pesticides drained into rivers after they were applied to the area near the rivers. Waters from major rivers, such as the Shinano River, are utilized for many purposes, including for drinking water. Therefore, it is important to investigate levels of pesticide contamination that may cause adverse effects, not only to people but also to the ecosystems near rivers.

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