Bull. Environ. Contam. Toxicol. (1996) 57:442-449

Bulletin of Environmental Contamination and Toxicology

Pesticides in Water and Fish from Rivers Flowing into Lake Biwa

T. Tsuda, T. Inoue, M. Kojima, S. Aoki

Shiga Prefectural Institute of Public Health and Environmental Science, 13-45, Gotenhama, Ohtsu, Shiga 520, Japan

Received: 21 October 1995/Accepted: 20 March 1996

Pesticide contamination of surface waters from agriculture use is a problem of worldwide importance. Many field data on the pesticide contamination of surface waters and aquatic organisms have been reported in Japan (Araki and Hayakawa 1995; Environment Agency 1993; 1994; Fukushima 1991; Murakami et al. 1990; Watanugi et al. 1993).

We have already reported various pesticide contamination of water and fish (pale chub, ayu sweetfish and dark chub) from rivers flowing into Lake Biwa from April in 1988 to March in 1992 (Tsuda et al. 1991; 1992a; 1994). Pesticides in the surveys were insecticides (diazinon, fenitrothion, malathion, salithion, methyl parathion, chlorpyriphos, fenthion, parathion, isofenphos, phenthoate, prothiophos, propaphos and methidathion), fungicides (IBP, tolclofos-methyl and edifenphos) and herbicides (benthiocarb, simetryne, oxadiazon, CNP, chlomethoxynil and butamifos). From the field data, it has become apparent that the contamination of fish by pesticides in the field can be approximately estimated from the laboratory accumulation data on fish (Kanazawa 1981; Tsuda et al. 1988; 1989; 1992b).

In this report, the same surveys were more extensively performed for twenty one pesticides (salithion, diazinon, IBP, tolclofos-methyl, chlorpyriphos, fenthion, malathion, fenitrothion, isofenphos, phenthoate, prothiophos, propaphos, methidathion, butamifos, isoprothiolane, edifenphos, EPN, pyridaphenthion, phosmet, benthiocarb and simetryne) from April in 1992 to March in 1993, and for nine pesticides (fenobucarb, carbofuran, simazine, chlorothalonil, pretilachlor, isoprotiolane, flutolanil, benthiocarb and symetrine) from April in 1993 to March in 1994.

Correspondence to: T. Tsuda

NATERIALS AND METHODS

Salithion, diazinon, IBP, tolclofos-methyl, chlorpyriphos, fenthion, malathion, fenitrothion, isofenphos, phenthoate, prothiophos, propaphos, methidathion, butamifos, isoprothiolane, edifenphos, EPN, pyridaphenthion, phosmet, benthiocarb, simetryne, fenobucarb, carbofuran, simazine, chlorothalonil, pretilachlor, flutolanil were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). These chemicals containing more than 98.0% in ingredient were used without further purification. Activated charcoal was Darco G60 from Wako Pure Chemical Industries Ltd. Microcrystalline cellulose was AVICEL for column chromatography from FUNAKOSHI Co Ltd (Tokyo, Japan). Solvents were pesticide grade and other chemicals were reagent grade.

Water and fish samples were collected in seven rivers flowing into Lake Biwa once or twice every month from April in 1992 to March in 1994. Sampling locations were the same as in our previous surveys (Tsuda et al. 1991; 1992a; 1994). Fish samples were pale chub (*Zacco platypus*, body length 6.1 - 9.6 cm and body weight 3.7 - 14.9 g), ayu sweetfish (*Plecoglossus altivelis*, body length 6.2 - 8.7 cm and body weight 2.9 - 8.0 g) and dark chub (*Zacco temminckii*, body length 4.5 - 7.6 cm and body weight 1.5 - 7.6 g). Water samples were immediately analyzed. Fish samples were homogenized as a mixture of three or four whole body samples for each sampling location and frozen and preserved for analysis.

The concentration of each pesticide in the water was determined by the following procedure. A measured volume (1000 ml) of the water was shaken with 100 ml of dichloromethane after addition of 50 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 hexane. Determination of the pesticides in the hexane solution was performed using selected ion monitoring (SIM) by gas chromatography-mass spectrometory (GC-MS). Average recoveries (n=3) were 87 - 100 % at 1.0 ng/ml spiked levels of the twenty-seven pesticides and detection limits were 0.01 ng/ml for tolclofos-methyl, fenthion, isofenphos, prothiophos, propaphos, EPN and phosmet, 0.02 ng/ml for salithion, diazinon, IBP, chlorpyriphos, malathion, fenitrothion, phenthoate, methidathion, butamifos, isoprothiolane, edifenphos, benthiocarb, simetryne, fenobucarb, carbofuran, pretilachlor and flutolanil, 0.05 ng/ml for pyridaphenthion, 0.1 ng/ml for chlorothalonil and 0.2 ng/ml for simazine. Determination of the pesticides in the fish samples was performed by the same method as in our previous report (Tsuda et al. 1994). Detection limits were 5 - 10 ng/g for salithion, diazinon, IBP, tolclofos-methyl, chlorpyriphos, fenthion, malathion, fenitrothion, isofenphos, phenthoate, prothiophos, propaphos, methidathion, butamifos, isoprothiolane, edifenphos, EPN, pyridaphenthion, phosmet by FPD-GC and 5 ng/g for fenobucarb, carbofuran and simetryne, 10 ng/g for simazine, chlorothalonil, pretilachlor and benthiocarb and 20 ng/g for flutolanil by FTD-GC. Operating conditions of GC-MS (Finnigan mat MAGNUM), FPD-GC (Shimadzu GC-SAM) and FTD-GC (Shimadzu GC-14B) were as follows:

GC-MS

Ion mode: EI Manifold temperature: 220°C Multiplier voltage: 1800 V
Emission current: 10 µA
GC column: J&W DB-1701 (0.53 mmϕ x 30 m, film thickness 1.0 µm)
Temperatures: injection 250°C; column 60°C (1 min) 10°C/min to 200°C (0 min) 2°C/min to 220°C (0 min) 10°C/min to 270°C (10 min)
Carrier: He 10 ml/min

FPD-GC

GC column: ULBON HR-1701 (0.32 mmϕ x 25 m, film thickness 0.25 µm)
Carrier: He 1 ml/min Air: 60 ml/min Hz: 75 ml/min
Temperatures: injection and detector 280°C; column 50°C (2 min) 10°C/min to 200°C (0 min) 2°C/min to 220°C (0 min) 10°C/min to 270°C (10 min)

FTD-GC

GC column: J&W DB-1701 (0.53 mmϕ x 30 m, film thickness 1.0 µm)
Carrier: He 20 ml/min Air: 150 ml/min Hz: 3.5 ml/min
Temperatures: injection and detector 280°C; column 150°C (1 min) 5°C/min to 250°C (5 min)

Calculation of BCF

BCF was calculated by the following equation:

$$BCF = \frac{\text{chemical concentration in whole body of fish}}{\text{chemical concentration in water}}$$

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

RESULTS AND DISCUSSION

From the survey of the twenty one pesticides in river water from April in 1992 to March in 1993, ten pesticides (diazinon, IBP, simetryne, benthiocarb, chlorpyriphos, fenthion, fenitrothion, isoprothiolane, edifenphos and EPN) were detected by GC-MS. GC-MS determination of the pesticides was performed using SIM with ions at m/z=179 for diazinon, m/z=204 for IBP, m/z=213 for simetryne, m/z=100 for benthiocarb, m/z=197 for chlorpyrifos, m/z=278 for fenthion, m/z=277 for fenitrothion, m/z=118 for isoprothiolane, m/z=109 for edifenphos and m/z=157 for EPN, respectively.

These pesticides in river water were detected at high frequency: simetryne (56/98), chlorpyriphos (42/98), isoprothiolane (61/98) and benthiocarb (23/98) but at low frequency for diazinon (3/98), IBP (9/98), fenthion (3/98), fenitrothion (4/98), edifenphos (6/98) and EPN (2/98). Simetryne was detected from May to January (0.05 - 59.8 ng/ml) and at high concentrations in May in the seven rivers. Chlorpyriphos was detected all the year round (0.01 - 0.14 ng/ml) in the seven rivers. Isoprothiolane was also detected all the year round (0.02 - 12.3 ng/ml) in the four rivers and at high concentrations in August. The concentrations of the other pesticides were 0.03 - 0.05 ng/ml from Apri 1 to August for diazinon, 0.02 - 0.22 ng/ml from July to August for IBP, 0.01 - 0.87 ng/ml from May to July for benthiocarb, 0.01 - 0.04 ng/ml in August for fenthion, 0.03 - 0.21 ng/ml from Apri 1 to August for fenitrothion, 0.05 - 0.75 ng/ml in August for edifenphos and 0.05 ng/ml in January and March for EPN. An example of the concentration changes of the ten pesticides in the water and fish (Senjyo River) is shown in Figure 1 throughout the survey from April in 1992 to March in 1993. Detection of isoprothiolane in the dark chub corresponded well to that in the water. Simetryne was detected in the dark chub only on June 3 in spite of the detection on May 28, June 3 and June 25 in the river water. This is probably due to the low bioconcentration potential of simetryne in the dark chub from the laboratory experimental data BCF=2 on willow shiner (Tsuda et al. 1988). Detection of benthiocarb (9.9 ng/g) in the dark chub from the river water (0.04 ng/ml) was reasonable from the same data BCF=65 on willow shiner (Tsuda et al. 1988) and BCF=170 on topmouth gudgeon (Kanazawa 1981). No detections of chlorpyriphos, fenitrothion and edifenphos in the dark chub in spite of their detections in the river water are probably due to

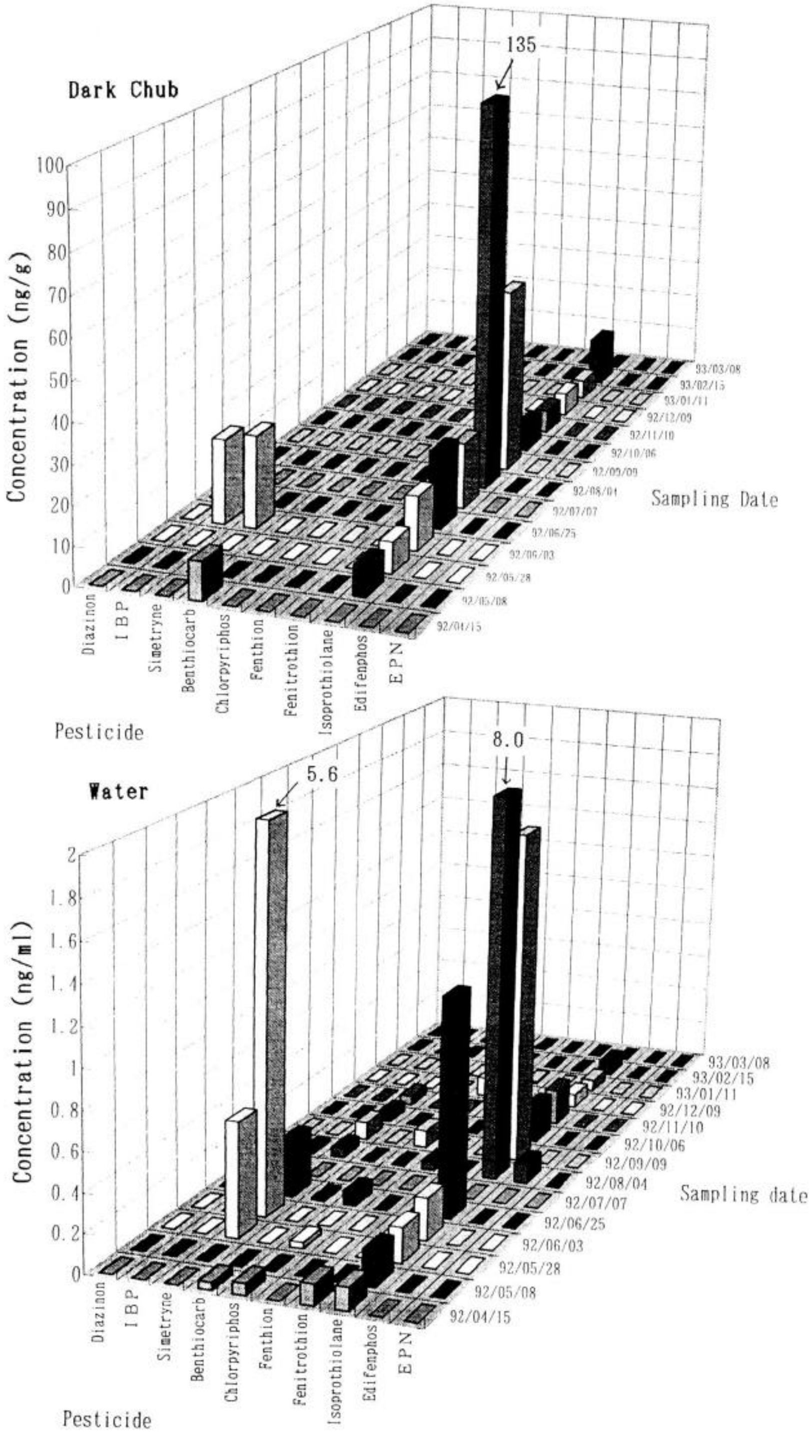

Figure 1. Concentration changes of ten pesticides in water and dark chub obtained from Senjyo River from April in 1992 to March in 1993.

their temporary contamination or low bioconcentration potential.

From the survey of the nine pesticides in the river water from April in 1993 to March in 1994, eight pesticides (fenobucarb, carbofuran, simazine, pretilachlor, isoprothiolane, flutolanil, simetryne, benthiocarb) were detected by GC-MS. GC-MS determination of the pesticides was performed using SIM with ions at m/z=121 for fenobucarb, m/z=164 for carbofuran, m/z=201 for simazine, m/z=176 for pretilachlor, m/z=118 for isoprothiolane, m/z=173 for flutolani1, m/z=213 for simetryne and m/z=100 for benthiocarb, respectively. These pesticides in the river water were detected at high frequency for simetryne (72/105), isoprothiolane (61/105), carbofuran (34/105), flutolanil (32/105), benthiocarb (32/105), simazine (27/105) and fenobucarb (24/105) but at low frequency for pretilachlor (11/105). Simetryne was detected from May to February (0.02 - 208 ng/ml) and at high concentrations in May in the seven rivers. Isoprothiolane was detected al 1 the year round (0.04 - 1.8 ng/ml) in the three rivers and at high concentrations in July and August. Benthiocarb was detected from May to September (0.02 - 14.8 ng/ml) and at high concentrations in May in the seven rivers. The results of simetryne, isoprothiolane and benthiocarb in this survey were nearly equal to those in the preceding year. The concentrations of the other pesticides were 0.04 - 1.1 ng/ml in May and June for carbofuran, 0.04 - 0.11 ng/ml from May to February for flutolanil, 0.2 - 1.7 ng/ml from May to October for simazine, 0.04 - 0.09 ng/ml from May to October for fenobucarb and 0.04 - 5.1 ng/ml in May for pretilachlor. Detection of isoprothiolane, simetryne and benthiocarb in the fish samples from the river waters had the same tendency as those in the preceding year. No detections of carbofuran and pretilachlor in the fish samples in spite of their detections in the river waters are probably due to their low bioconcentration potential.

The average BCF values of the pesticides in pale chub, ayu sweetfish and dark chub were calculated from the field data and summarized in Table 1 as field BCF data together with laboratory BCF data (Tsuda et al. 1988; 1989; Kanazawa 1981; Tsuda et al. unpublished). The field BCF values of simetryne 2 - 20 and benthiocarb 56 - 248 in the three species of fish were nearly equal to the laboratory BCF values of simetryne 2 in willow shiner and benthiocarb 65 - 382 in the three species of fish, respectively. However, the field BCF values IBP 165 (n=l) in pale chub and fenobucarb 148 (n=1) in ayu sweetfish were considerably higher than the laboratory BCF values of IBP 4 - 33 in the three species of fish and fenobucarb 26 in topmouth gudgeon, respectively. This is probably because the concentrations of these pesticides in the river water decreased rapidly and those in the fish bodies did not reach plateaus instantly. There were no wide differences between the field BCF data and the laboratory BCF data. More accurate evaluation of the field BCF data will be possible in case of larger numbers of field data. Further, the difference of fish species should be considered for the evaluation of the field BCF data by the laboratory BCF data.

Table 1. Comparison of field BCF data with laboratory BCF data

Pesticides	Field BCF data			Laboratory BCF data		
	Pale chub	Ayu sweetfish	Dark chub	Willow shiner[a]	Topmouth gudgeon[b]	killifish[c]
IBP	165(n=1)	-	-	33	4	9
Simetryne	7(n=7)	20(n=6)	2(n=2)	2	-	-
Benthiocarb	68(n=8)	56(n=7)	248(n=1)	65	170	382
Isoprothiolane	233(n=19)	226(n=18)	173(n=19)	-	-	45
Fenobucarb	-	148(n=1)	-	-	26	-
Carbofuran	-	112(n=1)	-	-	-	-
Pretilachlor	19(n=1)	-	-	-	-	-

a Data from Tsuda et al. (1988, 1989)
b Data from Kanzawa (1981)
c Unpublished data from Tsuda et al.

REFERENCES

Araki K, Hayakawa S (1995) Survey of pesticide in the river water. Rept Environ Sci Inst Mie Pref 15: 87 - 89 (in Japanese)

Environment Agency of Japan (1993) Chemicals in the Environment (in Japanese)

Environment Agency of Japan (1994) Chemicals in the Environment (in Japanese)

Fukushima M (1991) Fate and behavior of pesticide in aquatic environment - Case study of Yodo River Basin - . Jpn J Water Pollut Res 14: 79 - 83 (in Japanese)

Kanazawa J (1981) Measurement of the bioconcentration factors of pesticides by freshwater fish and their correlation with physicochemical properties or acute toxicities. Pestic Sci 12: 417 - 424

Murakami Y, Fukushima S, Nishimune T, Sueki K, Tanaka R (1990) Examination of pollution by organochlorine herbicides and fungicides in Yodo River and Osaka Bay. J Food Hyg Soc Japan 31: 36 - 43 (in Japanese).

Moriguchi Y, Shiode S, Takenaka K, Fukushima M, Fujita T, Yamaguchi Y (1994) Suido Suigen niokeru Nouyaku no Bunseki to Jittai. Journal of Japan Water Works Assoc 63: 28 - 37 (in Japanese)

Tsuda T, Aoki S, Kojima M, Fujita T (1994) Pesticides in water and fish from rivers flowing into Lake Biwa (III). Toxicol Environ Chem 41: 85 - 90.

Tsuda T, Aoki S, Kojima M, Fujita T (1992a) Pesticides in water and fish from rivers flowing into Lake Biwa (II). Chemosphere 24: 1523 - 1531.

Tsuda T, Aoki S, Kojima M, Fujita T (1992b) Accumulation and excretion of organophosphorous pesticides by willow shiner. Chemosphere 25: 1945 - 1951.

Tsuda T, Aoki S, Kojima M, Harada H (1991) Pesticides in water and fish from rivers flowing into Lake Biwa. Toxicol Environ Chem 34: 39 - 55.

Tsuda T, Aoki S, Kojima M, Harada H (1990) Accumulation and excretion of oxadiazon, CNP and chlomethoxynil by willow shiner. Comp Biochem Physiol 96C: 373 - 375.

Tsuda T, Aoki S, Kojima M, Harada H (1989) Bioconcentration and excretion of diazinon, IBP, malathion and fenitrothion by willow shiner. Toxicol Environ Chem 24: 185 - 190.

Tsuda T, Aoki S, Kojima M, Harada H (1988) Bioconcentration and excretion of benthiocarb and simetryne by willow shiner. Toxicol Environ Chem 18: 31 - 36.

Watanugi F, Namiki T, Tsukabayashi H (1993) Fates of paddy herbicides in water course and residues in fishes. Rent Ishikawa Pref lnst Pub Hlth & Environ Sci 30: 121 - 130 (in Japanese)