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Pesticides and Their Oxidation Products in Water and Fish from Rivers Flowing into Lake Biwa

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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 1994 (Tsuda et al. 1991: 1992a; 1994; 1996a). Pesticides in the surveys were insecticides (diazinon, fenitrothion, malathion, salithion, methyl parathion. chlorpyriphos, fenthion, parathion, isofenphos, phenthoate, prothiophos, propaphos, methidathion, EPN, pyridaphenthion, phosmet, isoprothiolane, fenobucarb and carbofuran), fungicides (IBP, tolclofos-methyl, edifenphos and flutolanil) and herbicides (benthiocarb, simetryne, oxadiazon, CNP. chlomethoxynil, butamifos, simazine, chlorothalonil and pretilachlor). 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).

Recently, the contamination of surface waters by oxidation products of pesticides such as ketomolinate, fenthion sulfoxide, fenthion sulfone, disulfoton sulfone and isoprothiolane sulfoxide has been reported in the Mississippi River in the USA (Pereira and Hostettler 1993) and Yodo River Basin in Japan (Yamaguchi and Fukushima 1994; Moriguchi et al. 1994). However, few reports (Tsuda et al. 1996b; 1997) have been published concerning the accumulation and excretion of these oxidation products of pesticides in the field and the laboratory aquatic organisms.

In this report, surveys of molinate, disulfoton, fenthion, isoprothiolane and their oxidation products (2-ketomolinate, disulfoton sulfoxide, disulfoton sulfone, fenthion sulfoxide, fenthion sulfone and isoprothiolane sulfoxide) were performed for water and fish samples obtained from seven rivers flowing into Lake Biwa from April in 1995 to March in 1996.

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MATERIALS AND METHODS

Molinate (S-ethyl perhydroazepin-1-carbothionate), 2-ketomolinate (S-ethylperhydroazepin-1-carbothioate-2-one), disulfoton (0,0-diethyl S-2-ethyl thioethyl phosphorodithioate), disulfoton sulfoxide (0,0-diethyl S-2-ethylsulfoxoethyl phosphorodithioate), disulfoton sulfone (0,0-diethyl S-2-ethylsulfonylethyl phosphorodithioate) and isoprothiolane sulfoxide (diisopropyl 1-oxo-1,3-dithiolan-2-ylidenemalonate) were purchased from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan), and fenthion (0,0-dimethyl-0-4-mehtylthio-m-tolyl phosphorothioate), fenthion sulfoxide (0,0-dimethyl-0-4-methylsulfinyl-m-tolyl phosphorop thioate), fenthion sulfone [0,0-dimethyl-0-(3-mehtyl-4-methylsulfonylnhenyl) phosphorothioatel and isoprothiolane (diisopropyl 1,3-dithiolane-2-ylidene-malonate) from Wako Pure Chemical Industries Ltd (Osaka, Japan). These chemicals containing more than 99% in ingredient were used without further purification. Sen-Pak Florisil column (Waters, USA) was used for sample purification. 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 1995 to March in 1996 except for December. Sampling locations were the same as in our previous surveys (Tsuda et al. 1991: 1992a; 1994; 1996a). Fish samples were pale chub (*Zacco platypus*, body length 5.4 - 9.1 cm and body weight 4.6 - 14.9 g), ayu sweetfish (*Plecoglossus altivelis*, body length 6.4 - 9.0 cm and body weight 3.3 - 10.3 g) and dark chub (*Zacco temminckii*, body length 4.8 - 7.2 cm and body weight 2.2 - 7.1 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.

Each concentration of the chemicals in water samples was determined by the following procedure. A measured volume (1000 ml) of 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 chemicals in the hexane solution was performed using selected ion monitoring (SIM) by gas chromatography-mass spectrometry (GC-MS), by flame photometric gas chromatography (FPD-CC) and by electron capture gas chromatography (ECD-CC). Average recoveries (n=3) were 100% for molinate, 105% for 2-ketomolinate, 87% for disulfoton, 110% for disulfoton sulfoxide, 91% for disulfoton sulfone. 100% for fenthion. 70% for fenthion sulfoxide, 86% for fenthion sulfone, 90% for isoprothiolane, 110% for isonrothiolane sulfoxide at a spiked level of 0.5 ng/ml. Detection limits were 0.002 ng/ml for disulfoton and disulfoton sulfoxide. 0.005 ng/ml for fenthion, 0.01 ng/ml for molinate, disulfoton sulfone, fenthion sulfoxide, fenthion sulfone and isoprothiolane, 0.02 ng/ml for isoprothiolane sulfoxide and 0.05 ng/ml for 2-ketomolinate. Determination of the chemicals in fish samples was performed by the method of Goto and Kato (1980) modif ied as follows. In brief, fish sample (ca 5 g) was homogenized twice with each 30 ml acetonitrile after addition of 5 g anhydrous Na_2SO_4 and the organic layer was filtered. The combined filtrate was shaken with 15 ml hexane and the acetonitrile layer was rotary-vacuum evaporated just to dryness. The residue was dissolved with 5ml of hexane and passed through a Sep-pak Florisil column. Each chemical was eluted with 20 ml of acetone+hexane (2+8) after washing with 10 ml of hexane. The eluate was analyzed by GC-MS, FPD-GC and ECD-GC after evaporation to 1 ml under nitrogen stream. Average recoveries (whole body of fish 5 g, n=3) were 60% for molinate, 90% for 2-ketomolinate, 70% for disulfoton, 74% for disulfoton sulfoxide, 87% for disulfoton sulfone, 78% for fenthion, 79% for fenthion sulfoxide, 85% for fenthion sulfone, 80% for isoprothiolane, 72% for isoprothiolane sulfoxide at a spiked level of 100 ng/g. Detection limits were 0.5 ng/g for disulfoton and disulfoton sulfoxide, 1.0 ng/g for fenthion, 2.0 ng/g for molinate, disulfoton sulfone, fenthion sulfoxide, fenthion sulfone, 5.0 ng/g for 2-ketomolinate and isoprothiolane and 10.0 ng/g for isoprothiolane sulfoxide. Operating conditions of GC-MS (Finnigan mat MAGNUM), FPD-GC (Shimadzu GC-14B) and ECD-GC (Shimadzu GC-15A) 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: J&W DB-5 (0. 25 mmϕ x 30 m, film thickness 0.25 µm)
Carrier: He 1 ml/min Air: 60 ml/min H_2: 75 ml/min
Temperatures: injection and detector 300°C; column 60°C (0 min) 20°C/min to 200°C (0 min) 2°C/min to 230°C (0 min) 25°C/min to 270°C (5 min)

ECD-GC
GC column: J&W DB-1 (0.53 mmϕ x 30 m, film thickness 1.0 µm)
Carrier: N_2 20 ml/min Temperatures: injection and detector 250°C; column 220°C

Calculation of BCF

BCF was calculated by the following equation:

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

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 this survey in river water from April in 1995 to March in 1996, four pesticides (molinate. disulfoton, fenthion and isoprothiolane) and their oxidation products (2-ketomolinate, disulfoton sulfoxide, disulfoton sulfone, fenthion sulfoxide, fenthion sulfone, isoprothiolane sulfoxide) were detected by GC-MS, FPD-GC and ECD-GC. GC-MS determination was performed using SIM with ions at m/z=126 for molinate, m/z=186 for disulfoton, m/z=278 for fenthion, m/z=162 for isoprothiolane, m/z=202 for 2-ketomolinate, m/z=125 for disulfoton sulfoxide, m/z=213 for disulfoton sulfone, m/z=279 for fenthion sulfoxide, m/z=310 for fenthion sulfone and m/z=247 for isoprothiolane sulfoxide, respectively.

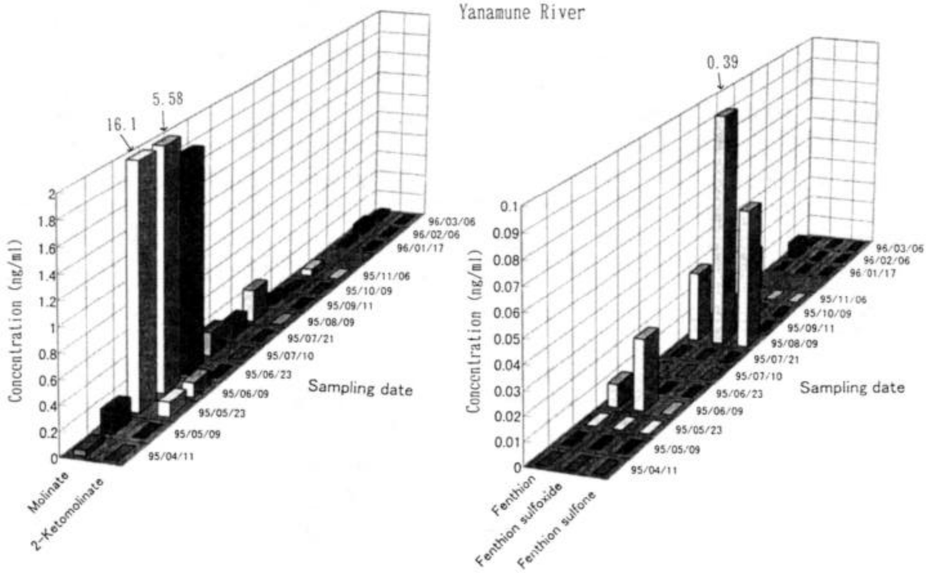

Figure 1. Concentration changes of molinate, fenthion and their oxidation products in water obtained from Yanamune River from April in 1995 to March in 1996.

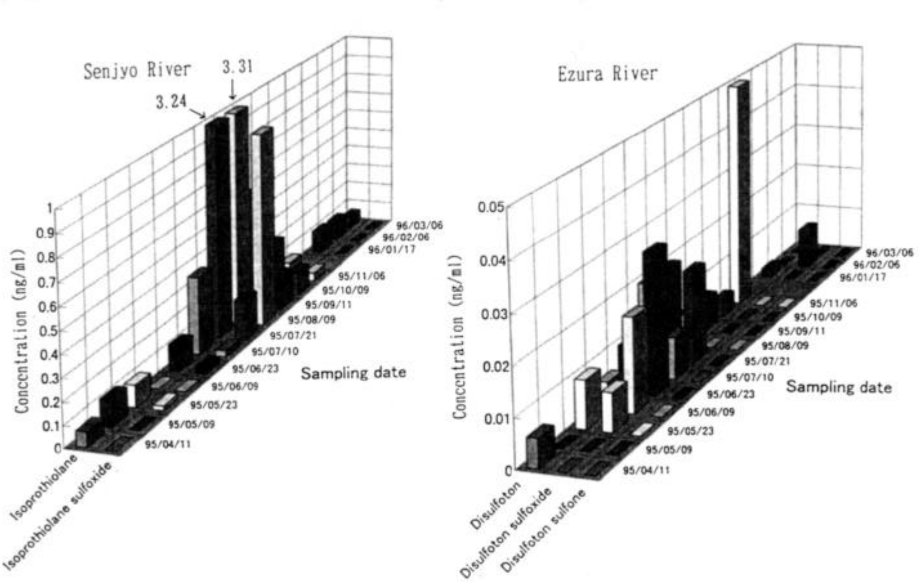

Figure 2. Concentration changes of isoprothiolane, disulfoton and their oxidation products in water obtained from Senjyo River and Ezura River from April in 1995 to March in 1996.

Table 1. Proportions of oxidation products in pesticides for water samples from rivers flowing into Lake Biwa

Oxidation product/Pesticide	No. of data	Range (%)	Average (%)
2-Ketomolinate/Molinate	4	0.7 - 2.2	1.1
Disulfoton sulfoxide/Disulfoton	17	20.0 - 12.00 X100	2.22 X100
Disulfoton sulfone/Disulfoton	3	65.0 - 48.00 X100	17.88 X100
Fenthion sulfoxide/Fenthion	5	33.3 - 17.78 X100	7.42 X100
Fenthion sulfone/Fenthion	3	33.3 - 3.33 X100	1.89 X100
Isoprothiolane sulfoxide/Isoprothiolane	26	3.3 - 4.00 X100	36.1

Molinate was detected all the year round (0.01 - 19.5 ng/ml) and at high concentrations in May - June in the four rivers. 2-Ketomolinate was detected in May - June (0.06 - 0.13 ng/ml) in the three rivers. 2-Ketomolinate was detected at lower frequency (4/98), compared with molinate (37/98). Disulfoton was detected from April to January (0.002 - 0.2 ng/ml) in the seven rivers. Its oxidation products were detected from April to February (0.002 - 0.49 ng/ml) in the six rivers for disulfoton sulfoxide and from June to August (0.03 - 0.24 ng/ml) in the three rivers for disulfoton sulfone. Disulfoton sulfone was detected at lower frequency (4/98), compared with disulfoton (38/98) and disulfoton sulfoxide (33/98). Fenthion was detected mainly from June to August (0.005 - 0.12 ng/ml) in the six rivers. Its oxidation products were detected from June to August (0.02 - 0.42 ng/ml) in the five rivers for fenthion sulfoxide and in August (0.03 - 0.06 ng/ml) in the three rivers for fenthion sulfone. Frequency of detection was 11/98. 6/98 and 3/98 for fenthion, fenthion sulfoxide and fenthion sulfone, respectively. Isoprothiolane was detected all the year round (0.01 - 6.24 ng/ml> and at high concentrations in July - August in the seven rivers. Isoprothiolane sulfoxide was detected from May to November (0.02 - 0.91 ng/ml) in the six rivers. Isoprothiolane sulfoxide was detected at lower frequency (26/98), compared with isoprothiolane (62/98). For all pesticides in this survey, the oxidation products were detected at lower frequency. compared with the original pesticides. Examples of the concentration changes of these pesticides and their oxidation products in the water are shown in Figure 1 (Yanamune River) and Figure 2 (Senjyo River and Ezura River) throughout the survey from April in 1995 to March in 1996.

From the data in this survey, proportions of oxidation products in original pesticides were calculated and are shown in Table 1. The proportions of 2-ketomolinatelmolinate and isoprothiolane sulfoxide/isoprothiolane were 1.1 % (average, n=4) and 36.1 % (average, n=26), respectively. The values were considerably lower than those of disulfoton sulfoxideldisulfoton 2.22 x 100 % (average, n=17), disulfoton sulfone/disulfoton 17.88 × 100 % (average, n=3), fenthion sulfoxide/fenthion 7.42 × 100 % (average, n=5) and fenthion sulfone/fenthion 1.89 × 100 % (average, n=3). It was presumed that disulfoton and fenthion were more rapidly oxidized than molinate and isoprothiolane or the oxidation products of disulfoton and fenthion were more stable than those of molinate and isoprothiolane in the environment.

An example of the concentration changes of the pesticides and their oxidation products in the water and fish (Ezura River) is shown in Figure 3 throughout the survey from April in 1995 to March in 1996. In this Figure, no data were shown at the dates of 95/4/11, 95/7/21, 95/9/11, 95/10/9, 95/11/6, 96/1/17 and 96/2/6 for Pale chub because of its no collections in these sampling dates. Detections of molinate, 2-ketomolinate and fenthion in the pale chub corresponded well to those in the water. No detections of fenthion sulfoxide (<2.0 ng/g) and isoprothiolane (< 5.0 ng/g) in the fish in spite of their detections in the river water (fenthion sulfoxide 0.42 ng/ml, isoprothiolane 0.03 - 0.73 ng/ml) are probably due to their temporary contamination or low bioconcentration potential from the laboratory BCF data (fenthion sulfoxide 1.7. isoprothiolane 45) in killifish (Tsuda et al 1996b; 1997).

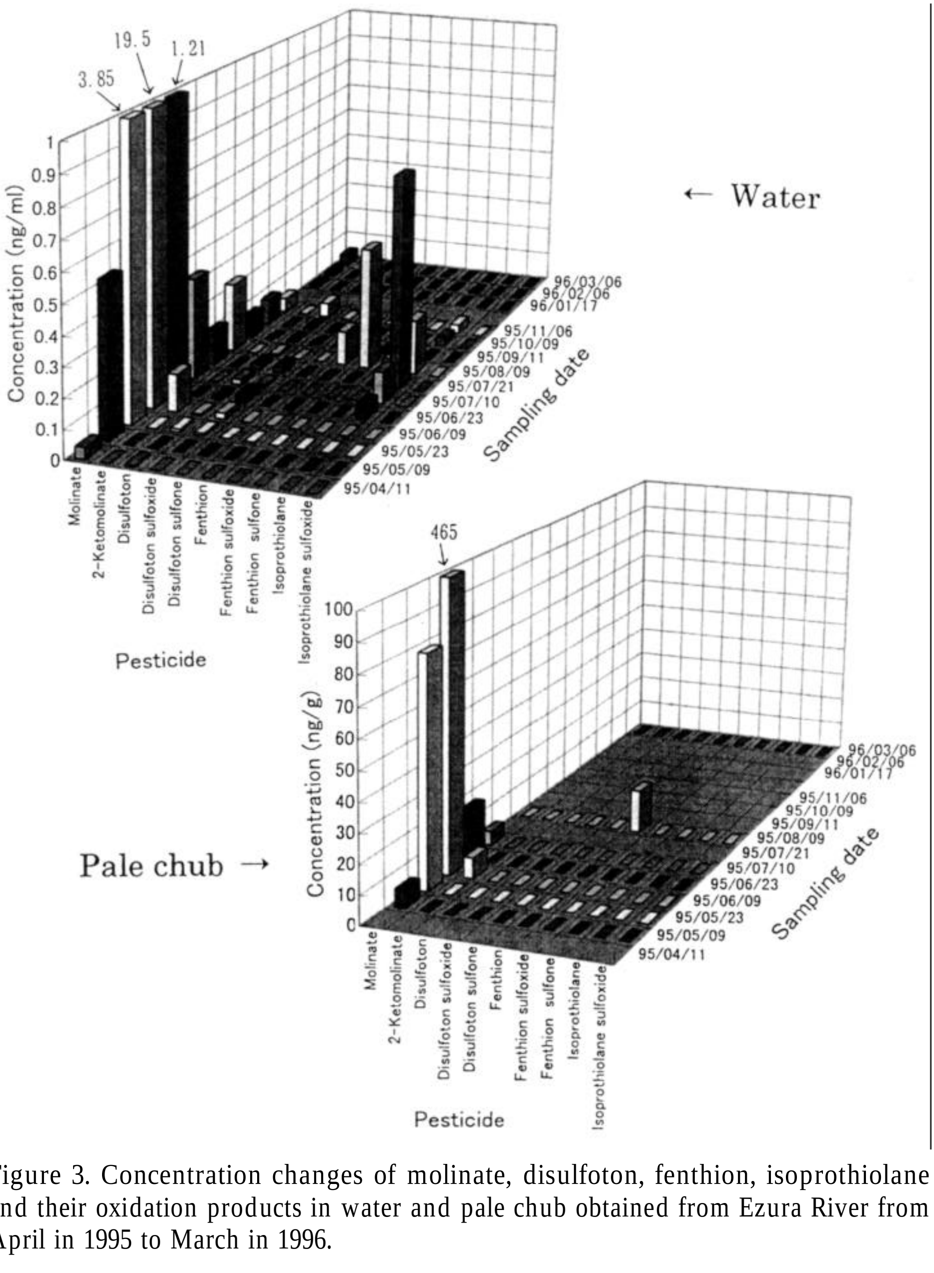

Figure 3. Concentration changes of molinate, disulfoton, fenthion, isoprothiolane and their oxidation products in water and pale chub obtained from Ezura River from April in 1995 to March in 1996.

Table 2. Comparison of field BCF data with laboratory BCF data

Chemicals	Field BCF data			Laboratory BCF data		
	Pale chub	Ayu sweetfish	Dark chub	Willow shiner[a]	Topmouth gudgeon[b]	Killifish[c]
Molinate	16 (n=5)	13 (n=4)	–	–	26	–
2-Ketomolinate	55 (n=1)	–	–	–	–	–
Disulfoton	–	–	–	–	–	–
Disulfoton sulfoxide	–	–	–	–	–	–
Disulfoton sulfone	–	–	–	–	–	–
Fenthion	125 (n=1)	–	–	481	–	154
Fenthion sulfoxide	–	–	–	–	–	1.7
Fenthion sulfone	–	–	–	–	–	3.2
Isoprothiolane	156 (n=4)	153 (n=4)	272 (n=4)	–	–	45
Isoprothiolane sulfoxide	–	–	–	–	–	0.2

[a] Data from Tsuda et al. (1992b)

[b] Data from Kanazawa (1981)

[c] Data from Tsuda et al. (1996b, in press)

The average BCF values of the pesticides and their oxidation products in pale chub, ayu sweetfish and dark chub were calculated from the field data and are summarized in Table 2 as field BCF data together with laboratory BCF data (Tsuda et al. 1992b; 1996b: 1997; Kanazawa 1981). The field BCF values of molinate 16 in pale chub and 13 in ayu sweetfish and fenthion 125 in pale chub were nearly equal to the laboratory BCF values of molinate 26 in topmouth gudgeon and fenthion 154 in killifish. However, the field BCF values of isoprothiolane 153 - 272 in the three species of fish were higher than the laboratory BCF value of 45 in killifish. The results were almost the same as those in our previous surveys (Tsuda et al. 1991; 1992a; 1994; 1996a). For oxidation products of the pesticides, their BCF values were calculated only for 2-ketomolinatc but not for the oxidation products of disulfoton, fenthion and isoprothiolane because of their no detections in fish. This is probably due to their low bioconcentration potential from the laboratory BCF data in killifish (fenthion sulfoxide 1.7, fenthion sulfone 3.2 and isoprothiolane sulfoxide 0.2).

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