Bull. Environ. Contam. Toxicol. (1992) 48:474–480

Environmental Contamination and Toxicology

Accumulation and Release of Herbicides Butachlor, Thiobencarb, and Chlomethoxyfen by Fish, Clam, and Shrimp

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Butachlor (*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide), thiobencarb (benthiocarb, *S*-((4-chlorophenyl)methyl) diethylcarbamothioate) and chlomethoxyfen (chlomethoxynil, 2,4-dichloro-1-(3-methoxy-4-nitrophenoxy)benzene) are the most popular herbicides used to control weeds in transplanted rice paddy fields in Taiwan. Each of these herbicides are used alone or as part of a mixture. Applied to the paddies, these herbicides dissipate into paddy water and are absorbed into the soil (Beestman and Deming 1974; Chen and Chen 1979; Ishikawa *et al.* 1976). They disappear in irrigation water (Yusa and Ishikawa 1977), but still a minor amount of the herbicide could be detected in paddy drainage water even several weeks after application (Chiang *et al.* 1987). Herbicides applied to paddy fields may also flow out with effluents, causing contamination of river water (Yamagishi and Akiyama 1981; Ohyama *et al.* 1986; 1987).

Studies on bioconcentration and excretion of the herbicide by fish have been carried out by practical measurement after theoretical discussion (Ohyama *et al.* 1987; Tsuda *et al.* 1988; Schimmel *et al.* 1983; Neely 1979). To understand the accumulation and release of these three herbicides by fish and mussel in the aquatic environments at lower concentrations, experiments were performed by purposely exposing fish, clam and shrimp to the herbicide at a concentration of one hundredth and one thousandth of their respective 48-hr LC50 values.

MATERIALS AND METHODS

Pure compounds for reference standards in analysis were obtained from Monsanto Co., U. S. A. (butachlor, 99.2%), Kumiai Chemical Industry Co., Ltd., Japan

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(thiobencarb, 100%) and Ishihara Sangyo Co., Ltd., Japan (chlomethoxyfen, 99.9%). Technical grade herbicides were also provided from the above mentioned companies, respectively. They were butachlor of 90%, thiobencarb of 93% and chlomethoxyfen of 85% purity. Carp (*Cyprinus carpio* T. & S.) 3.5 to 4.0 cm in size, tilapia (*Oreochromis mossambicus*) 3.5 to 4.0 cm, loach (*Misgurnus anguillicaudatus*) 5.0 to 6.5 cm, grass carp (*Ctenopharyngodon idullus*) 3.5 to 4.0 cm, eel (*Anguilla japonica* T. & S.) 15 to 20 cm, black silver carp (*Aristichthys nobilis*) 5.0 to 6.5 cm, freshwater clam (*Corbicula fluminea* Muller) 1.3 to 2.0 cm and macrobranch shrimp (*Macrobrachium rosenbergii*) 4.0 to 5.0 cm were all used as test organisms, cultivated at the Chu-Pei Fish Culture Station, Taiwan Fisheries Research Institute, Chu-Pei, Hsin-Chu, Taiwan, Republic of China. The test fish was acclimatized for 1 wk in conditions similar to those under which the test are to be performed. They were fed once a day during the acclimatization period and were not be fed for a period of 2 d before they were used in a test.

LC50 values were evaluated by the methods described by Nishiuchi (1974). The experiments were carried out at a temperature of 22+1 °C. Technical grade herbicide was dissolved in a definite volume of acetone to make a stock solution, and then diluted with a large volume of clean water (pH: 6.6; DO: 4.9 mg/L without aeration and 6.7 mg/L with aeration; hardness: 215 mg/L as $CaCO_3$). The weight of all the fish in a test container was not exceeded 1 g/L of the water. Experiments were performed with aeration only in the case of shrimp. A concurrent control was performed in exactly the same manner using only 0.02% by volumn of acetone and clean water without herbicide. If the mortality exceeded 10% during the control test, the results were omitted. Plotting the test concentration vs survival percentage, 48-hr LC50 values were derived by the straight line graphical interpolation method introduced by Nichiuchi (1974). Long-term bioconcentration studies were conducted individually on three herbicides, with concentrations of approximately one hundredth and one thousandth (one fiftieth and one hundredth for carp and eel) of their respective 48-hr LC50 values. For each study, fish were placed in a 500-L plastic tank that received clean water amended with herbicide. The aqueous solution was renewed every 2 d. Uptake and depuration tests were continued for 30 d. Depuration test was performed by removing the experimental fish, 3 d after the uptake test, from their exposure tank to another tank receiving clean water. During the uptake and depuration portions of each test, six fish were randomly sampled from the experimental tank on 0, 1, 3, 5, 7, 10, 15, 20, 25 and 30 d, and analyzed in triplicate, individually. Bioconcentration factor

(BCF) were calculated from the maximum accumulation of the herbicide concentration in fish divided by the herbicide concentration in water.

About 10 g of fish sample (5 g for clam) were homogenized with 25 mL of *n*-hexane and 25 mL of acetonitrile and filtered. The residue was washed with the same solvent twice more. The *n*-hexane layer of the above mixed solvent was separated and extracted thrice with 150 mL of acetonitrile. The acetonitrile portion was collected and evaporated with rotary evaporator to a small volume and passed through a column (6 x 0.8 cm id) packed with Al_2O_3 and eluted with 150 mL of acetonitrile saturated with *n*-hexane. The eluate was measured by ECD-GC. The average recoveries were 92.35, 89.61 and 93.11% for butachlor, thiobencarb and chlomethoxyfen, respectively.

ECD gas chromatography was performed with Shimadzu GC-7A Gas Chromatograph. A glass column (2 m x 3 mm id) with 3% OV-1 on 80/100 mesh Chromosorb WHP was employed for analysis of butachlor and chlomethoxyfen. For analysis of thiobencarb, the liquid phase of the packing material was replaced by 3% OV-17. Operating temperatures were as follows: injection port, 250 °C; detector, 280 °C; column, 215 °C for analysis of butachlor and thiobencarb, 225 °C for analysis of chlomethoxyfen. Nitrogen was used as a carrier gas.

RESULTS AND DISCUSSION

Owing to the slight solubility of chlomethoxyfen in water, its LC50 value could not be determined. When the shell was closed tightly, it was not easy to know if clams were alive or not. When clams were exposed to water with butachlor and thiobencarb up to 20 mg/L individually, still no LC50 value could be obtained in this study. Macrobranch shrimp showed a higher tolerance than all other organisms, except for clams. The 48-hr LC50 values are shown in Table 1.

Table 1. 48-Hr LC50 value (mg/L)

Organism	Butachlor	Thiobencarb
Carp	0.93	1.93
Tilapia	0.88	1.99
Loach	0.89	2.54
Grass carp	0.24	1.51
Eel	0.29	0.89
Black silver carp	0.58	2.45
Freshwater clam	> 20	> 20
Macrobranch shrimp	7.71	3.47

Maximum accumulation in organisms and the BCF at long-term test are shown in Table 2. Maximum accumulations were reached within 3 to 5 d after exposure to

Table 2. Maximum accumulation (within 3 to 5-d expoure) in organisms and the bioconcentration factor (BCF)

Organism	Herbicide in water, μg/L	Maxi.accumu.in organism, μg/L	BCF
A. Butachlor			
Carp	18.0	195	10.8
	9.0	51	5.7
Tilapia	10.0	24	2.4
	1.0	7	7.0
Loach	10.0	28	2.8
	1.0	7	7.0
Grass carp	2.5	143	57
	1.25	133	106
Eel	5.0	255	51
	2.5	198	79
Black silver carp	2.4	235	98
	0.4	88	220
Freshwater clam	100.0	112	1.1
	10.0	60	6.0
Macrobranch shrimp	100.0	0.56(7 d)	0.01
	10.0	0.30(10 d)	0.03
B. Thiobencarb			
Carp	37.2	2160	58
	18.6	718	39
Tilapia	20.0	487	24
	2.0	21	10
Loach	20.0	613	31
	2.0	27	13.5
Grass carp	15.0	124	8.3
	7.5	90	12.0
Eel	10.0	159	16
	5.0	92	18
Black silver carp	5.0	1,876	375
	1.4	1,223	874
Freshwater clam	200.0	570	2.9
	20.0	145	7.3
Macrobranch shrimp	50.0	0.27(15 d)	0.01
	5.0	0.03(15 d)	0.01
C. Chlomethoxyfen			
Tilapia	30.0	3,180	106
Loach	30.0	4,660	155
Grass carp	5.0	475	95
	2.5	74	30
Eel	5.0	8,680	1,736
	2.5	11,770	4,708
Black silver carp	17.8	6,953	391
	2.0	2,217	1,109
Freshwater clam	30.0	1,730	58
Macrobranch shrimp	20.0	251	13
	2.0	26	13

Table 3. Bioconcentration and excretion of herbicides butachlor, thiobencarb and chlomethoxyfen by organisms

Organism	Herbicide concentration (μg/L) in water	in fish		
	Prefeeding 3 d	Removed to clean water 0 d	5 d	30 d
A. Butachlor				
Carp	10.0	44	14.0	1.6
	1.0	6.7	3.5	2.6
Tilapia	10.0	25	9.6	1.8
	1.0	6.6	3.8	1.8
Loach	10.0	29	10.4	3.1
	1.0	7.0	4.4	1.9
Grass carp	2.5	124	ND	ND
	1.25	96	ND	ND
Eel	5.0	205	ND	ND
	2.5	171	ND	ND
Black silver carp	2.4	475	334	85
	0.4	247	219	92
Freshwater clam	100.0	132	14.0	3.0
	10.0	62	10.3	0.9
Macrobranch shrimp	100.0	0.1	ND	ND
	10.0	0.1	ND	ND
B. Thiobencarb				
Carp	20.0	1,263	446	33
	2.0	24	9.9	4.6
Tilapia	20.0	432	37	2.8
	2.0	20	4.1	ND
Loach	20.0	285	32	5.4
	2.0	13.5	3.7	0.9
Grass carp	15.0	88	ND	ND
	7.5	74	ND	ND
Eel	10.0	129	ND	ND
	5.0	92	ND	ND
Black silver carp	5.0	3,687	1,900	900
	1.4	1,721	1,160	358
Freshwater clam	200.0	582	126	18
	20.0	130	19	2.4
Macrobranch shrimp	50.0	ND	ND	ND
	5.0	ND	ND	ND
C. Chlomethoxyfen				
Tilapia	30.0	2,420	490	180
Loach	30.0	3,830	1,520	250
Grass carp	5.0	475	127	9.0
	2.5	74	15	ND
Eel	5.0	3,670	1,030	60
	2.5	2,760	490	40
Black silver carp*	17.8	8,482	111	50
	2.0	2,278	72	29
Freshwater clam	30.0	1,580	640	80
Macrobranch shrimp	20.0	227	49	ND
	2.0	21	4.0	ND

ND: <0.01 μg/L. * Prefeeding for 14 d.

herbicide, except for macrobranch shrimp (7 to 10 d for butachlor and 15 d for thiobencarb), and then kept at this level throughout the experiment. In macrobranch shrimp, maximum accumulation of herbicide was lowest, and the time to maximum accumulation was longest. The bioconcentration factor at maximum accumulation level in macrobranch shrimp was only 0.01 (in 100 μg/L of herbicide) and 0.03 (10 μg/L) for butachlor, 0.01 (50 μg/L) and 0.01 (5 μg/L) for thiobencarb, and 13 (20 μg/L) and 13 (2 μg/L) for chlomethoxyfen. The extremely low value of BCF for macrobranch shrimp may be attributed to its biological speciality, or due to some kinds of special enzymes presented in the shrimp. Black silver carp showed the largest BCF for all the three herbicides.

The results of depuration tests are shown in Table 3. More than half of the herbicide residue in carp, tilapia, loach, clam and black silver carp was excreted within 5 d in clean water; only a slight residue was found after 30 d. In macrobranch shrimp, grass carp and eel, almost negligible or nondetectable residue was found within 3 d in clean water. Schimmel *et al.* (1983) reported that some pesticides were depurated by oysters to nondetectable concentration within 1 wk after termination of exposure in pesticide. The results by Tsuda *et al.* (1988) also revealed that herbicides benthiocarb and simetryne were rapidly excreted from fish after their removal to clean water for 2 to 7 d.

Acknowledgments. This study was supported by the Council of Agriculture of Republic of China, 76-Agr-3.1-fish-31, 77-Agr-7.1-fish-28, 78-Agr-7.1-fish-35 and 79-Agr-7.1-fish-13.

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Received June 10, 1991; accepted October 30, 1991.