

Acute Toxicity, Accumulation, and Excretion of Benthioncarb and Its Degradation Products in Killifish

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Pesticide contamination of surface waters from agricultural use is a problem of worldwide importance. Much field data on the pesticide contamination of surface waters and aquatic organisms have been reported in Japan (Araki et al. 1993, 1994; Araki and Hayakawa 1995; Environment Agency 1993; 1994; 1995; Fukushima 1991; Murakami et al. 1990; Nagafuchi et al. 1994; Takahashi et al. 1994; Tsuda et al. 1991; 1992; 1994; Watanugi et al. 1993). 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). Further, degradation of various pesticides has been investigated in waters containing residual chlorine and ozone (Inoue et al. 1995; Magara et al. 1994; Ohto et al. 1993; Okumura 1992; Takahashi and Morita 1993). However, few reports (Tsuda et al. 1996a) have been published concerning the accumulation and excretion of these degradation products of pesticides in the field and the laboratory aquatic organisms.

As a measure of toxicity, mutagenicity tests have been done for organo-oxophosphorous (P=O compounds prepared from their corresponding organo-thiophosphorous (P=S) pesticides (Onodera et al. 1995) and for degradation products of benthioncarb (Kume et al. 1996) and isoprothiolane (Inoue et al. 1995) which are produced by chlorination during water purification of raw water for tap water supply. However, no reports have been published for acute or chronic toxicity of these degradation products to fish

Therefore, we have performed laboratory tests of acute toxicity, accumulation and excretion, using killifish for representative pesticides and their degradation products which are detected at high frequency in river water (Yamaguchi and Fukushima 1994) or produced by chlorination during water purification of raw water for tap water supply (Inoue et al. 1995; Kume et al. 1996). In the present study, we report the results of these experiments for benthioncarb and its degradation products.

MATERIALS AND METHODS

Benthioncarb [$\text{ClC}_6\text{H}_4\text{CH}_2\text{SCON}(\text{C}_2\text{H}_5)_2$], purity 99.0 %, 4-chlorobenzyl alcohol ($\text{ClC}_6\text{H}_4\text{CH}_2\text{OH}$),

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purity 99.9 %, and 4-chlorobenzaldehyde ($\text{ClC}_6\text{H}_4\text{CHO}$), purity 99.7 % were purchased from Hayashi Pure Chemical Industries Ltd (Osaka Japan) and used without further purification.

Killifish (*Oryzias latipes*) were purchased from a local pet shop. The body length and body weight of the fish were respectively 2.3 - 2.6 cm and 0.23 - 0.33 g in acute toxicity test, and 1.9 - 2.3 cm and 0.15 - 0.26 g in accumulation and excretion tests. All fish were acclimated to water of the quality to be used in the tests for 7 days before use. A commercial assorted feed was given at 2.5 mg/fish once a day in the accumulation and excretion tests, and until 48 hr before testing in the acute toxicity test. Benthioncarb and its degradation products were not detected in the fish before exposure to these chemicals.

For acute toxicity tests, three aqueous stock solutions of benthioncarb (10 mg/L), 4-chlorobenzyl alcohol (50 mg/L) and 4-chlorobenzaldehyde (50 mg/L) were prepared by dissolving weighed amounts of the chemicals in the dilution water which was prepared by aerating city water overnight. Test solutions were prepared by diluting appropriate aliquots of stock solutions with the dilution water. For accumulation and excretion tests, three aqueous stock solutions of benthioncarb (800 $\mu\text{g/L}$), 4-chlorobenzyl alcohol (400 $\mu\text{g/L}$) and 4-chlorobenzaldehyde (400 $\mu\text{g/L}$) were prepared by diluting ethanol solutions of benthioncarb (2000 $\mu\text{g/mL}$), 4-chlorobenzyl alcohol (2000 $\mu\text{g/mL}$, 2 mL), and 4-chlorobenzaldehyde (2000 $\mu\text{g/mL}$, 2 mL) with dechlorinated city water to 10 L, respectively.

Acute toxicity tests were carried out according to OECD Guideline for Testing of Chemicals (1981). Killifish were exposed to a range of concentration of benthioncarb, 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde (each 1 L, of test solution in beakers) for 48 hr under static conditions at $21 \pm 1^\circ\text{C}$. The concentrations tested were 1.5, 2.4, 3.9, 6.3 and 10 mg/L for benthioncarb, 7.6, 12.2, 19.5, 31.3 and 50 mg/L for 4-chlorobenzyl alcohol and 1.5, 2.4, 3.9, 6.3 and 10 mg/L for 4-chlorobenzaldehyde. Duplicates, each containing 5 fish, were used for the control and each concentration of these chemicals. After 24 and 48 hr the fish were assessed for loss of swimming ability. The pH and the concentrations of dissolved oxygen in each test water were 7.3 - 7.4 and 5.8 - 6.5 mg/L, respectively. Measurements were carried out every day.

Accumulation and excretion tests were carried out without aeration under a continuous flow-through system. Experimental conditions were as reported in Tsuda et al. (1987). Glass tanks holding ca. 10 and 5 L were used for the accumulation and excretion tests, respectively.

For the accumulation tests (96 hr), benthioncarb (800 $\mu\text{g/L}$), 4-chlorobenzyl alcohol (400 $\mu\text{g/L}$) and 4-chlorobenzaldehyde (400 $\mu\text{g/L}$) were respectively diluted 25 times with dechlorinated city water and supplied by continuous flow to each of three aquaria containing 250 fish each. During the test the flow rate and temperature of water for each exposure were maintained at 3.0 L/hr and $20 \pm 1^\circ\text{C}$, respectively. The concentration of the chemicals in each exposure tank were [mean \pm SD (n+6)] 11.0 ± 1.1 $\mu\text{g/L}$ for benthioncarb

(experiment 1), 7.1 ± 0.5 $\mu\text{g/L}$ for 4-chlorobenzyl alcohol (experiment 2) and 4.4 ± 0.6 $\mu\text{g/L}$ for 4-chlorobenzaldehyde (experiment 3). Measurements of chemical concentrations in each exposure tank were carried out at 0, 6, 12, 24, 48 and 96 hr, respectively. Twenty fish were taken at 6, 12, 24, 48 and 96 hr, respectively. All of the fish were frozen and preserved for analysis after rinsing with distilled water.

The concentrations of these chemicals in each exposure tank were adjusted to 1/6000 - 1/400 of the data of 48-hr LC_{50} killifish (Table 1). The pH, the concentrations of dissolved oxygen, and the hardness in each exposure water were 6.7-6.8, 6.7 -7.0 mg/L and 43-45 mg/L as CaCO_3 , respectively. Measurements were carried out every day for pH and after 24 hr for dissolved oxygen and hardness.

For the excretion tests (12 hr), about 100 fish remaining in each aquarium were separately transferred into each of the other three aquaria and dechlorinated city water was supplied to each aquarium. During the tests, the flow rate and temperature of water in each aquarium were maintained at 6 L/hr and $20 \pm 1^\circ\text{C}$, respectively. Twenty fish were taken at 3, 6 and 12 hr, respectively. All of the fish were frozen and preserved for analysis after rinsing with distilled water. The pH, the concentrations of dissolved oxygen, and the hardness in each test water were 6.7-6.8, 7.1 -7.3 mg/L and 43-44 mg/L as CaCO_3 , respectively. Measurements were carried out after 6 hr for pH, dissolved oxygen and hardness.

Under these conditions, none of the fish showed any signs of lethargy or agitation during the accumulation and excretion tests.

Each concentration of benthocarb, 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde in water samples was determined by the following procedure. A measured volume (100 mL) of water was shaken with 50 mL of hexane after addition of 20 g of NaCl. The organic layer was concentrated to 5 mL with a rotary evaporator at 40°C . The hexane solution was injected into a gas chromatograph unit equipped with an electron capture detector (ECD-GC). Average recoveries ($n=3$) were 82 % for benthocarb, 81 % for 4-chlorobenzyl alcohol and 84 % for 4-chlorobenzaldehyde at a spiked level of 5 $\mu\text{g/L}$.

Determination of the chemicals in fish samples was carried out by the method of Goto and Kato (1980) modified as follows. In brief, a fish sample (ca 2 g) was homogenized twice with 30 ml of acetonitrile after addition of 3 g of anhydrous Na_2SO_4 and the organic layer was filtered. The combined filtrate was evaporated just to dryness for benthocarb, and concentrated to 1 mL after washing with 15 mL of hexane for 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde with rotary evaporator at 40°C . For benthocarb, the residue was dissolved in 10 mL of hexane and shaken twice with each 30 mL of acetonitrile saturated with hexane. The combined acetonitrile layer was evaporated just to dryness with the rotary evaporator. The residue was dissolved in 5 mL of hexane, passed through a Sep-Pak Plus Florisil column (Waters, USA) and eluted with 10 mL of acetone+hexane (1+9) after washing with 5 mL of hexane. The eluate was analyzed by ECD-GC after evaporation to 5 mL under nitrogen stream at 40°C . For 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde, the 1 mL solution was diluted with 5 mL of acetone+hexane (2+8), passed through a Sep-Pak Plus

Florisil column (Waters, USA) and further eluted with 5 mL of acetone+hexane (2+8). The eluate was concentrated to 1 mL with a rotary evaporator at 40°C and diluted with 5 mL of acetone+hexane (1+9). The solution was again passed through a Sep-Pak Plus Florisil column (Waters, USA) and further eluted with 5 mL of acetone+hexane (1+9). The eluate was analyzed by ECD-GC after evaporation to 5 mL under nitrogen stream at 40°C. Average recoveries (Whole body of fish 2 g, n=3) were 86 % for benthocarb, 99 % for 4-chlorobenzyl alcohol and 72 % for 4-chlorobenzaldehyde at a spiked level of 500 ng/g. The GC (Shimadzu GC-15A) operating conditions were as follows:

GC column: J&W DB-1 (0.53 mm ϕ x 30 m, film thickness 1.0 μ m)

Carrier: N₂ 20 ml/min Temperatures: injection and detector 280°C;

column 100°C (1 min) 5°C /min to 130°C (0 min) 20°C /min to 260°C (3 min)

The thawed fish samples were analyzed as a sample (each 20 fish) at each sampling time of accumulation and excretion tests.

Bioconcentration factor (BCF) was calculated from the following equation:

$$BCF = \frac{\text{chemical concentration in whole fish body (ng/g, wet weight)}}{\text{chemical concentration in water (}\mu\text{g/L)}}$$

The chemical concentration in the water at each sampling time was used for the calculation of BCF.

The following equation was used for the calculation of excretion rate constants of chemicals from the whole fish body.

$$C = C_0 e^{-kt}$$

where

C=chemical concentration in whole fish body (ng/g, wet weight) at time, t

C₀=initial chemical concentration in whole fish body (ng, wet weight)

k=first order excretion rate constant (hr⁻¹)

t=time (hr)

Partition coefficients between n-octanol and water (P_{ow}) of benthocarb and its degradation products were separately measured 3 times by flask-shaking method according to OECD Guideline for Testing of Chemicals (1981). Measurements were carried out for n-octanol/water (volume ratio) = 1 and 1/10. n-Octanol phase was diluted by ethanol, and analyzed by ECD-GC. Water phase was similarly analyzed after extraction. P_{ow} was calculated by the following equation: P_{ow} = chemical concentration in n-octanol/chemical concentration in water.

RESULTS AND DISCUSSION

The experimental results are shown in Table 1. The values of 24-hr LC₅₀ and 48-hr LC₅₀ were 10 and 4.1 mg/L for benthocarb, 58 and 44 mg/L for 4-chlorobenzyl alcohol and 7.6 and 7.6 mg/L for 4-chlorobenzaldehyde, respectively. The value of 48-hr LC₅₀ 4.1 mg/L for benthocarb was almost equal to that of 4.4 mg/L to killifish (Tanaka, 1978). For the degradation

products of benthocarb, the data of acute toxicity to fish were not reported in the literature. Acute toxicity of 4-chlorobenzyl alcohol to killifish (48-hr LC_{50} = 44 mg/L) was considerably lower than that of benthocarb (48-hr LC_{50} = 4.1 mg/L) but the toxicity of 4-chlorobenzaldehyde (48-hr LC_{50} = 7.6 mg/L) was nearly equal to that of benthocarb.

Table 1. Acute toxicity of benthocarb and its degradation products to killifish

Chemicals	LC_{50} (mg/L)	
	24 hr	48 hr
Benthocarb	10	4.1
4-Chlorobenzyl alcohol	58	44
4-Chlorobenzaldehyde	7.6	7.6

The experimental results are shown in Table 2. The BCF values of benthocarb degradation products (4-chlorobenzyl alcohol and 4-chlorobenzaldehyde) reached plateaus in 6-12 hr more rapidly than that of benthocarb (48 hr). The average BCF values of 4-chlorobenzyl alcohol (20, n=5) after 6- 96 hr and 4-chlorobenzaldehyde (2.2, n=4) after 12-95 hr were much lower than that of benthocarb (295, n=2) after 48 - 96 hr. The BCF values of benthocarb and its degradation products were approximately consistent with the P_{ow} values shown in Table 3. Ishikawa et al. (1977) reported that 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde were the major degradation products of benthocarb under ultraviolet lights or sunlight. Benthocarb was detected in river water from May to July in 1993 (0.02 - 14.8 μ g/L) in the survey of pesticides in rivers flowing into lake Biwa (Tsuda et al. 1996b). Degradation products of benthocarb were not measured in this survey, but 4-chlorobenzyl alcohol and 4-chlorobenzaldehyde were presumed to be detected in the rivers. From the low BCF values of 4-chlorobenzyl alcohol (20) and 4-chlorobenzaldehyde (2.2) in the killifish, their contamination of fish in the rivers was probably low.

Table 2. BCF of benthocarb and its degradation products in killifish

Chemicals	BCF in the whole killifish body				
	6 hr	12 hr	24 hr	48 hr	96 hr
Benthocarb	155	164	231	291	298
4-Chlorobenzyl alcohol	17	19	21	27	18
4-Chlorobenzaldehyde	0.7	2.6	2.3	2.2	1.8

Table 3. P_{ow} of benthocarb and its degradation products

Chemicals	$\log_{10} P_{ow}^a$	
	$O/W^b = .1$	$O/W = 1/10$
Benthocarb	3.86 \pm 0.07	4.26 \pm 0.08
4-Chlorobenzyl alcohol	2.27 \pm 0.11	1.93 \pm 0.09
4-Chlorobenzaldehyde	2.13 \pm 0.02	2.12 \pm 0.01

^aMean \pm SD (n=3)

^bn-Octanol/water

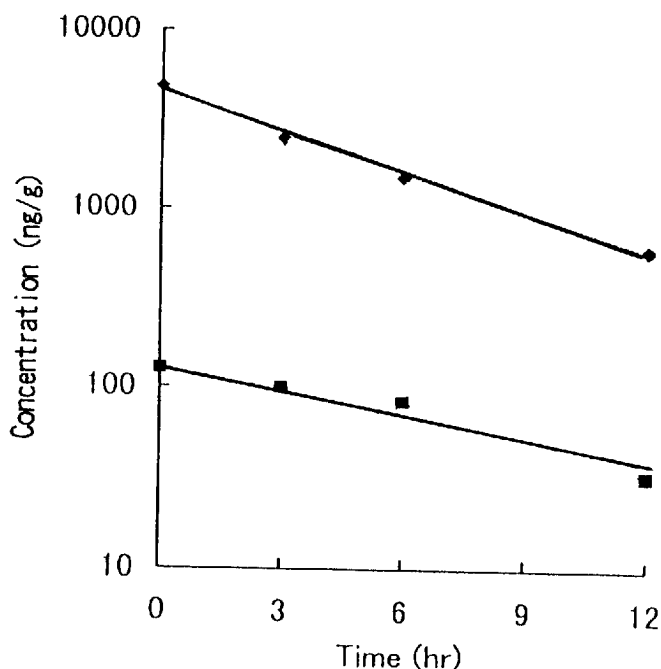


Figure 1. Excretion of benthocarb \blacklozenge and 4-chlorobenzyl alcohol \blacksquare from the whole killifish body.

The excretion of benthocarb and 4-chlorobenzyl alcohol from the killifish is shown in Figure 1. The concentrations of the chemicals in the fish at hr 0 were equal to those at 96 hr in the accumulation tests. The concentration of 4-chlorobenzaldehyde in the fish was very low and rapidly decreased, so could not be measured by ECD-GC after 3-12 hr. The excretion rate constants (k) and biological half-lives were calculated for benthocarb and 4-chlorobenzyl alcohol, and are shown in Table 4. The excretion rate of 4-chlorobenzyl alcohol (half-life = 7.7 hr) was slightly slower than that of benthocarb (half-life = 3.6 hr). The rate of 4-chlorobenzaldehyde could not be measured, but was presumed to be more rapid than that of 4-chlorobenzyl alcohol. The residue of these degradation products in fish in the environment was presumed to be as low as that of benthocarb.

It was suggested that benthocarb degradation products (4-chlorobenzyl alcohol and 4-chlorobenzaldehyde) are safer than benthocarb in the water environment from the experimental results of their acute toxicity, accumulation and excretion.

Table 4. Excretion rate constants and biological half-lives of benthocarb and its degradation products by killifish

Chemicals	k (hr^{-1})	Half-life (hr)
Benthocarb	0.19	3.6
4-Chlorobenzyl alcohol	0.09	7.7
4-Chlorobenzaldehyde	> 0.09	< 7.7

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