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Degradation of chlorobenzuron in water and metabolism in fish

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Abstract. The degradation of pesticide 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea (CCU) in water and its accumulation and metabolism in fish were studied using HPLC method. The results of acute toxicity of both CCU and its metabolites were also reported. The research demonstrated that CCU degraded quickly in aerobic aquatic environment and primary metabolites of CCU were 4-chlorophenyl urea and ortho-chlorobenzoic acid. Microorganisms play an important role for the degradation of CCU in water. Both the parents compound and its metabolites were not lethal to fish in tested concentration. The accumulation of CCU in fish was similar to that of diflubenzuron. The esterase in fish liver which could metabolized CCU was identified. The primary enzymatic degradation products of CCU is the same as that in water.

Keywords: chlorobenzuron; degradation; toxicity; metabolism.

INTRODUCTION

Chlorobenzuron, or 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea (CCU) is a new insecticide developed in China. The high toxicity exhibited by CCU toward many insects indicates that the compound may be extensively used for insect control. Consequently it is especially important to study on the toxicity and behavior in environment. CCU is a structural analog of diflubenzuron (DFB), which has been used for many years in many countries (Booth, 1977; Ivie, 1978; Johnson, 1980; Metcalf, 1975; Opdycke, 1982b; Pimprikar, 1982; Saleem, 1987). The fate of DFB in water and soil, its toxicity to non-target organisms and the mechanisms of detoxification in insects have been interesting topics for scientists. The conclusion is that DFB was highly selective and highly toxic to pest and low toxic to mammalian. This study was carried out for CCU to demonstrate its degradation in water, acute toxicity to and accumulation and metabolism in fish. The results showed that its toxicity to and accumulation in fish and primary metabolic pathway of CCU were very similar to that of DFB.

MATERIALS AND METHODS

Materials

Grass carp was got from breed pond of the Institute of Hydrobiology. Fish fry, age≤ 48 h, was used for acute test.

Degradation of CCU in aerobic aquatic environment

Filtered lake water was used in experiments, in which, the concentration of CCU was 0.1 ppm. Magnetic stirring was adopted to keep the system aerobic. Water samples were taken at different times and extracted with organic solvents. Both CCU and its primary metabolites were determined quantitatively and qualitatively by HPLC. Comparing study was done at the same time between sterilized and non-sterilized lake water. The system was kept away from the light to prevent photodegradation and growth of algae.

Toxicity test

Acute toxicity test (96 h) was adopted. Test solutions were changed every day. Stock solutions of tested chemicals were dissolved in ethyl alcohol. The same amount of alcohol was applied to the control. There was no discernible effect to fish.

Measurement of bioconcentration factor of CCU

BCF_5 were measured when the exposure concentration was 5 ppb and 50 ppb, respectively. Fish samples were purified by column chromatography. Residues of CCU both in water and fish were determined by HPLC.

In vitro metabolism of CCU

The fishes were killed and the livers removed quickly, weighed, minced with scissors and homogenized with cold water in ice bath. The homogenate was centrifuged and the supernatant was kept in refrigerator for test. The reaction was started by adding 25.6μg CCU (in alcohol), the final concentration of which was 0.8 mg/L, and incubated for 5 h (25°C). Boiled supernatant was used as control. The amount of alcohol applied to the mixture had no appreciable effect on enzyme activity. Metabolite in the incubation mixture was extracted with organic solvents. The combined extract was evaporated to dry by rotary evaporator and the residue was dissolved in 0.5 ml dichloromethane for TLC. Standard of metabolite was chromatographed at the same TLC plate. The spot of sample which had the same R_f with CPU standard was scraped for HPLC analysis.

Chemicals

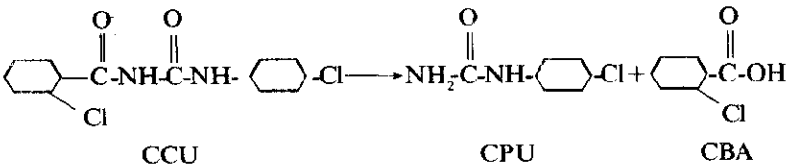
The CCU standard (> 95%) was provided by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Authentic standards of DFB (> 99%, Fluka, Switzerland), 4-chlorophenyl urea (CPU, > 99%, Merck, BRD), ortho-chlorobenzoic acid (CBA, > 99%, Merck, BRD), inhibitor profenofos (> 90.6%, Ciba Geigy, Switzerland), and TLC plate, silica gel 60 F_{254} (Merck, BRD) were gifts from Institute of Ecological Chemistry, GSF-Forschungszentrum fur Umweltund Gesundheit, BRD. Superpure water was prepared by Milli

Q water purification system (Millipore, America). Other chemicals were analytical reagent or chromatographic reagent.

RESULTS

Degradation of CCU in aerobic aquatic environment

Fig. 1 was a plotting of relative amount of CCU, CPU and CBA in the system vs time. It is obvious that CPU and CBA increased gradually as CCU decreased. At the end of 21 days CCU was only 20 percent of its initial amount. From the metabolites it can be concluded that primary degradation of CCU was the cleavage of N_1-C_1 bond, as follows:



CCU CPU CBA

This is the same degradation pathway as DFB. The results of UV scanning CPU standard and metabolite of CCU extracted from the system showed that the absorbance spectrum of both were coincidental (Fig. 2). Furthermore, the metabolite CBA was identified by characteristic mass spectra.

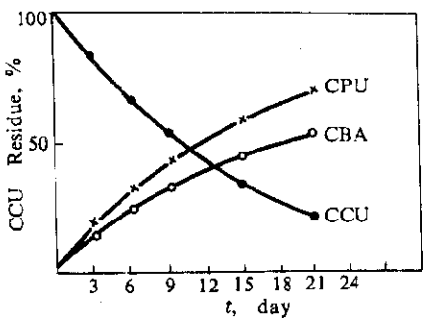


Fig. 1 Degradation of CCU in aerobic aquatic environment and formation of CPU and CBA

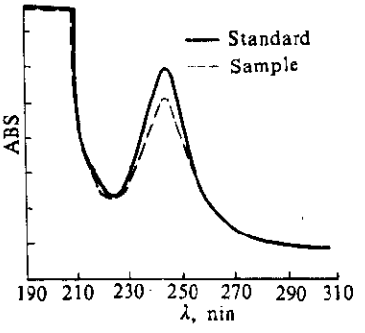


Fig. 2 UV absorbance spectrum of CPU standard and metabolits of CCU abs. AUFS: 0.05 scan AUFS: 0.10

Comparing the rate of chemical degradation and biodegradation of CCU

Fig. 3 shows CCU residue dynamic curve both in sterilized system and non-sterilized system. Decrease of CCU in non-sterilized system is much faster than that in sterilized system. The results fully proved the important role of microorganism in the degradation of CCU.

Acute toxicity of DFB, CCU and its metabolites to grass carp fry

Table 1 and Table 2 show the results of 96 h acute toxicity test. The experimental data indicated that DFB and CCU as well as metabolites of CCU had no acute toxicity to grass carp fry.

The solubility of CCU and DFB in water was 0.21 ppm and 0.23 ppm respectively. When the concentration of CCU and DFB was as high as 400 μg/L, no death of tested fish was observed. Primary metabolites of CCU, which had been proved in the tests, have higher polarity than parent compound and can dissolve in water more easily. Therefore, higher concentration was adopted in toxicity test than that done in CCU. The results demonstrated that primary metabolites of CCU in water had not presented acute lethal effect on grass carp fry even the concentration was as high as 2.5 ppm.

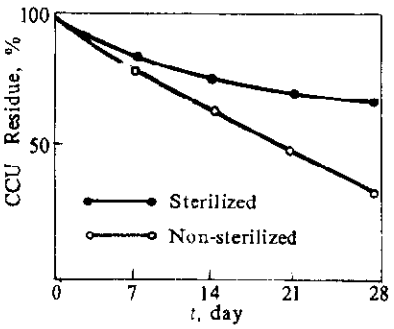


Fig. 3 Residue dynamics of CCU in lake water

Table 1 Survival rate of grass carp fry exposed to DFB and CCU, %

Concentration, μg/L	0	25	50	100	200	400
CCU	100	100	100	100	100	95
DFB	100	100	100	100	100	90

Table 2 Survival rate of grass carp fry exposed to CPU, CBA, %

Concentration, μg/L	0	2.5	25	250	2500
CPU	100	95	95	90	95
CBA	100	95	95	95	100

Bioconcentration factor (BCF) of CCU in fish

CCU has the property of lower water solubility and is lipophilic. Generally it should deposit in fish tissues and possess higher BCF. But the results were not the same as the prediction (Table 3). This indicated that there might be some mechanisms in fish, which were able to degrade CCU instantly.

Table 3 BCF_5 of fish exposed to CCU and DFB

Insecticide	Solubility, ppm	Exposure concentration, ppb	BCF_5
DFB	0.23	5	95.91
		50	119.3
CCU	0.21	5	59.69
		50	74.69

In vitro metabolism of CCU by enzyme of fish

There are various kinds of detoxification enzymes in animal liver. The previous studies indicated that at least three kinds of enzymes were contributing to the degradation of DFB in different animals (Metcalf, 1975; Opdycke, 1982; Pimprikar, 1982). Esterase was the major one of these enzymes. Hence the reaction medium of esterase was used in the test. Table 4 shows the result from in vitro test using fish liver preparation as enzyme. By using external standard and UV scanning it was confirmed that CPU was one of the primary enzymatic degradation products.

Table 4 The specific activity of enzyme in fish liver

Weight of fish, g	Liver, g	CPU, ng	CPU ng/g. liver/h
750	0.98	60.27	12.05
750	0.74	59.07	15.96
200 – 300	1.05	99.50	19.90
2000	0.82	74.43	17.18

Although there were differences in specific activities of different fish, which might be created by the variance of individual or homogenate in different tests, it showed the presence of enzymatic hydrolysis. There was no relationship between amount of CPU produced by fish and size of fish. That means grass carp did have ability to decrease CCU. This is the reason that lipophilic CCU was not accumulated highly in fish tissue. For insects the presence of such enzyme may create drug resistance.

The enzymes that metabolized DFB were also found in mammalian, birds, insects and fish (Booth, 1977; Ivie, 1978; Opdycke, 1982a; Pimprikar, 1979; Schaefer, 1979). For the non-target organisms of DFB, the presence of the enzymes could decrease accumulation of DFB in the body; nevertheless the presence of enzymes for target organisms may create drugresistance. So from the principle of enzymatic reaction, exploring and adding of synergist of insecticides are very important.

Identification of CCU degradation enzyme

Specific esterase inhibitor profenofos was added to reaction system to decide whether esterase was the key enzyme in enzymatic degradation of CCU or not. Table 5 shows the formation of CPU in the presence or asbsence of inhibitor. When inhibitor was used the production of CPU was almost fully inhibited. Apparently esterase plays an important role in the producing of CPU under test conditions. Cleavage of CCU in enzymatic degradation was the same as that in water.

Table 5 Inhibition of profenofos to the producing of CPU

	Enzyme, ng	Inhibitor, ng	Residue percentage, %
CPU	70.43	7.75	11
	103.43	1.00	1

SUMMARY

Benzoylphenyl urea insecticides are considered to disturb the ecdysis of insects by affecting the chitin metabolism. They will not interfere non-target organisms seriously because they possessed specific intoxication to pests. The results of degradation of chlorobenzuron were identical with general rule of this kind of insecticides. CCU degraded quickly in aquatic environment and formed the primary metabolites CPU and CBA, both the parents compound and its metabolites were not be found possessing the acute toxicity to grass carp. CCU had lower solubility in water, but not too high bioaccumulation factor in fish tissues, because there are at least one enzyme system which could metabolize CCU effectively in fish. It may be predicted that the chronic effects to fish will not happen, and that CCU is a safe insecticide possessing characteristics of non-persistent and low toxicity for non-target organisms.

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