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Calculation of the Peak Concentration of 2-Chlorobenzamide Generated in the Hydrolysis of the Benzoylphenylurea Insecticide 1-(2-Chlorobenzoyl)-3-(4-chlorophenyl)urea

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1-(2-Chlorobenzoyl)-3-(4-chlorophenyl)urea (CCU), a new analogue of diflubenuron and PH-6038, has been widely used in agriculture and forestry as a molt-inhibiting hormone insecticide which was developed in China. 2-Chlorobenzamide, a main degradation product of CCU in the environment, has been identified as a potential carcinogen, so the content of 2-chlorobenzamide from the breakup of CCU will directly affect the environmental safety of CCU. In this paper we describe a simple, rapid, and convenient prediction model for predicting the level and time of occurrence of the peak concentration of 2-chlorobenzamide in the hydrolysis of CCU verified by experimental data. The time for reaching the peak concentration of 2-chlorobenzamide (t_m) at 25 °C and pH 6 is 13.5 d, and the maximum concentration of 2-chlorobenzamide (y_m) is 3.2% of the initial concentration of CCU according to the results from the prediction model. These results are similar to the real values from the experiments, which are 22 d and 1.6% of the initial concentration of CCU, respectively. The difference between the values of the prediction and experiment is discussed, and it is demonstrated that the predicting model is highly credible.

KEYWORDS: 1-(2-Chlorobenzoyl)-3-(4-chlorophenyl)urea (CCU); 2-chlorobenzamide (CBA); degradation; predicting model

1. INTRODUCTION

Diflubenzuron has been known since the 1970s, and widely employed for protecting crops in the U.S. and Europe (*1*−*4*). It is one of the benzoylphenylurea (BPU) insecticides, and a lot of research work on its persistence and fate in plants and the environment has been done (*5*−*8*). PH-6038, an analogue of diflubenzuron, was synthesized as an insecticide after diflubenzuron, but it was not regarded as an applicable insecticide due to its persistence in the environment, so it was not spread once that was known. 1-(2-Chlorobenzoyl)-3-(4-chlorophenyl)-urea (CCU) is a new molt-inhibiting hormone insecticide which has a chemical structure similar to those of diflubenzuron and PH-6038 and was developed in China in the 1980s (*9*). CCU has been widely used in China in agriculture and forestry due to its advantages such as high effectiveness and low residual and no toxic effects on nontarget organisms (*10*). Nowadays, it is produced in a great quantity for the real agriculture and forestry need in two factories, Anyang Forestry Insecticide Factory and Tonghua Farm Insecticide Factory, located in North China and Northeast China, respectively. Some research focusing on its environmental behavior and toxicological characteristics had been done, and the results showed that 2-chlorobenzamide is a main degradation product produced from the degradations, i.e., photodegradation, hydrolysis, and microbial degradation, which probably occurred when CCU entered the environment (*11*−*13*). Moreover, 2-chlorobenzamide has been identified as a potential carcinogen by DNA adduct experiments (*14*). Therefore, the level of the peak concentration of 2-chlorobenzamide and its occurrence time became the most important factors to impact the environmental safety of CCU. Hydrolysis is one of the main pathways of the degradation of CCU, so it is of theoretical and practical value to establish a prediction model for 2-chlorobenzamide formation from the hydrolysis of CCU.

2. MATERIALS AND METHODS

2.1. Apparatus and Reagents. Quantitative analyses of CCU, 2-chlorobenzoic acid, and 2-chlorobenzamide were performed on a high-performance liquid chromatograph, Shimadzu model LC-6A (Japan), with a Shimadzu SPD-6AV UV−vis spectrophotometric detector and a 250 × 4.6 mm ZORBAX-C8 reversed-phase column. The mobile phase consisted of methanol and water mixed in a ratio of 7:3 (v/v), and the detection wavelength, flow rate, and injection volume were set at 254 nm, 0.4 mL/min, and 5 μL, respectively. A rotary vacuum evaporator (ZFQ-972, Tianjin, China) was employed for sample

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concentration. The standard samples of 2-chlorobenzamide and 2-chlorobenzoic acid were obtained from the Research Center of Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China. CCU was supplied by the Anyang Forestry Chemical Factory, which was used after being recrystallized three times with anhydrous ethanol.

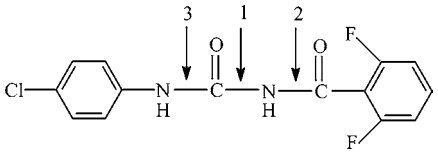
2.2. Methods. *2.2.1. Preparation of Stock Solutions*. CCU was weighed accurately to 500 ± 0.1 mg, added to nearly 100 mL of methanol under ultrasonication, then made up to 100 mL with methanol, and stored in the refrigerator for further use. A CBA stock solution was prepared in the same way with 493 ± 0.1 mg.

2.2.2. Procedure of Sample Pretreatment. After a set interval, 5 mL of hydrolytic solution was taken out and extracted with 3 × 5 mL of freshly distilled dichloromethane. The extracts were combined, dried with anhydrous Na_2SO_4, and then evaporated to near dryness with a rotary vacuum evaporator. The residue was dissolved in 0.5 mL of freshly distilled methanol for further analysis on HPLC.

2.2.3. Establishment and Verification of the Predication Model. The predication model about the level and occurrence time of the peak concentration of 2-chlorobenzamide in the hydrolysis of CCU was established by the analysis of the hydrolytic pathway of CCU, the establishment of the kinetic equation of every pathway, and the solution of the differential equation. The verification of the predication model was carried out by the comparison of the results obtained from the predication model and the experiment about the level and occurrence time of the peak concentration of 2-chlorobenzamide in the hydrolysis of CCU at pH 6.

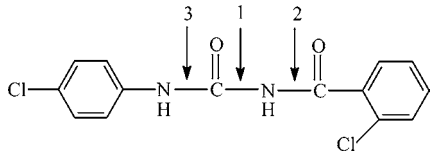
3. RESULTS AND DISCUSSION

3.1. Establishment of the Prediction Model. Diflubenzuron, 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea, is the first one developed for use in a series of benzoylphenylurea insecticides, and its chemical structure is as follows:



The hydrolysis of diflubenzuron has been investigated in detail (*15*, *16*), and the results showed that 2,6-diflorobenzoyl acid and (4-chlorobenzyl)urea were the main products owing to the breakup of the C−N bond at position 2 when the hydrolysis of diflubenzuron occurred.

CCU has the same basic molecular structure as diflubenzuron except that the substituted atom and number are different at the same phenyl ring. CCU has the following structure:



So CCU should undergo a degradation pathway similar to that of diflubenzuron in principle, and the products generated from the breakup of the C−N bond at position 2 should be the main products. However, a chlorine atom has a larger atomic semidiameter than a fluorine atom, which inhibited the cleavage of CCU at bond 2 and made it possible to break down bonds 1 and 3 to a certain degree because of steric interference. The study on the hydrolysis of CCU proved that the main degradation products were 2-chlorobenzoic acid, (4-chlorobenzyl)urea, 2-chlorobenzamide, and 4-chloroaniline (*12*), and the hydrolytic pathway can be depicted as in **Figure 1** (*17*).

From **Figure 1**, the hydrolytic rate constant *k* of CCU consists of two parts; one is the rate constant k_1 to produce 2-chlorobenzoic acid, and the other is the rate constant k_2 to produce 2-chlorobenzamide. These two degradation procedures occurred simultaneously in the environment; in other words, *k* was equal to the addition of k_1 and k_2. Besides the mentioned degradation absorbing more attention, another process was also very important for the safety of CCU; that is, the hydrolysis of 2-chlorobenzamide occurred when it formed from the breakup of CCU simultaneously, and k_3 was the hydrolytic rate constant. Therefore, if *a* was suggested as the original concentration of CCU and no 2-chlorobenzamide and 2-chlorobenzoic acid were present initially, meanwhile *x* and *y* were suggested as the concentrations of CCU and 2-chlorobenzamide after a *t* interval, eqs 1 and 2 were obtained.

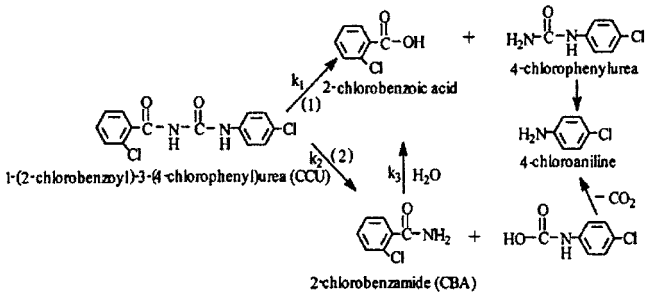


Figure 1. Hydrolytic pathway of CCU.

$$-\frac{dx}{dt} = k_1x + k_2x \tag{1}$$

$$\frac{dy}{dt} = k_2x - k_3y \tag{2}$$

From eq 1, eqs 3 and 4 were generated.

$$\ln\frac{a}{x} = (k_1 + k_2)t \tag{3}$$

$$x = ae^{-(k_1+k_2)t} \tag{4}$$

Substituting eq 4 into eq 2, eq 5 was obtained.

$$\frac{dy}{dt} = k_2ae^{-(k_1+k_2)t} - k_3y \tag{5}$$

Integration of eq 5 gave eq 6.

$$y = \frac{k_2a}{k_3 - (k_1 + k_2)}[e^{-(k_1+k_2)t} - e^{-k_3t}] \tag{6}$$

The peak concentration of 2-chlorobenzamide and the time to reach the peak concentration can be calculated with eqs 7 and 8 by computing an extreme value of eq 6.

$$t_m = \frac{\ln k_3 - \ln(k_1 + k_2)}{k_3 - (k_1 + k_2)} \tag{7}$$

$$y_m = \frac{k_2}{k_3}a\left(\frac{k_1 + k_2}{k_3}\right)^{(k_1+k_2)/[k_3-(k_1+k_2)]} \tag{8}$$

According to eqs 7 and 8, the level of the peak concentration of 2-chlorobenzamide and the time of the occurrence of the peak concentration can be predicted when the hydrolysis of CCU occurs in the environment.

3.2. Application of the Prediction Model. From eqs 7 and 8, as we have seen, the values of the kinetic rate coefficients k_1, k_2, and k_3 are crucial for predicting the level and occurrence

Table 1. Hydrolytic Rate Constants of CCU and 2-Chlorobenzamide at 25 °C

	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
CCU k (d^{-1})	0.01950	0.07895	0.1217	0.1492	0.1965	0.3650
CCU k (h^{-1})	0.000813	0.00329	0.00507	0.00621	0.00819	0.0152
CBA[a] k_3 (h^{-1})	0.00417	0.00286	0.00408	0.00109	0.00348	0.00411

[a] 2-Chlorobenzamide.

Table 2. Time for Reaching the Peak Concentration of 2-Chlorobenzamide at 25 °C (d)

	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
t_m		13.5	9.14	14.1	7.57	4.91

time of the peak concentration of 2-chlorobenzamide. In this study, the values of $k_1 + k_2$ and k_3 were obtained from the references, and the value of k_2 was determined from a series of experiments.

3.2.1. Values of $k_1 + k_2$ and k_3. Yang et al. has studied the hydrolytic behavior of CCU, and the values of the hydrolytic rate constant k (i.e., $k_1 + k_2$) of CCU in different pH buffer solutions has been reported in ref *12*. The hydrolytic rate constant k_3 of 2-chlorobenzamide in different pH buffer solutions has also been investigated in detail by our research group (*18*). The rate constant values of CCU and 2-chlorobenzamide are listed in **Table 1**.

3.2.2. Prediction of t_m. The values of k ($k_1 + k_2$) and k_3 in **Table 1** were applied to eq 7, and the time to reach the peak concentration of 2-chlorobenzamide in different pH water solutions was determined and is listed in **Table 2**. No data at pH 5 in **Table 2** were due to the fact that the hydrolytic rate constant of 2-chlorobenzamide was larger than that of CCU, which led to no store of 2-chlorobenzamide when CCU hydrolyzed at pH 5. Thus, a peak concentration would never be found under this condition.

3.2.3. Measurement of the Value of k_2. Besides the time of occurrence of the peak concentration, the level of the peak concentration was also of interest. However, it would never be obtained from eq 8 unless the k_2 value was obtained first. Hence, k_2 has become the bottleneck for predicting the level of the peak concentration of 2-chlorobenzamide in the hydrolysis of CCU. A series of experiments were designed and performed for the hydrolysis of CCU to obtain the rate constant k_2.

In the experiment, the reaction solution was prepared as follows: 0.5 mL of CCU stock solution was diluted to 5 mL with methanol, then transferred into about 500 mL of buffer solution (pH 6), and increased to 500 mL with the buffer. The buffer solution was obtained from the mixing of solutions of 250 mL of 0.1 M KH_2PO_4 and 28 mL of 0.1 M NaOH and by diluting to 500 mL. The reaction solution was kept in a thermostatic water bath, avoiding light, and the temperature was controlled at 25 °C. A 5 mL sample was removed from the reaction solution immediately, pretreated as described in section 2.2.2, and then analyzed by high-performance liquid chromatography (HPLC) to determine the initial concentration of CCU as 1.55×10^{-2} mmol/L. After 30 h, the residue of CCU and the degradation products were analyzed by HPLC. As we can see from **Figure 2**, four hydrolytic products have been detected and identified by comparing the retention times and the UV spectra of the analyte and the standard solutions as 2-chlorobenzoic acid, 2-chlorobenzamide, (*p*-chlorophenyl)urea, and *p*-chloroaniline. The retention times of the four products and

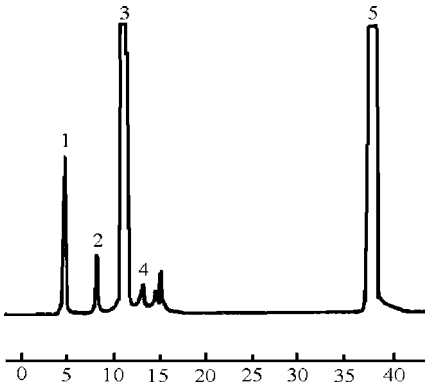


Figure 2. Chromatogram of CCU and its hydrolytic products over 30 h. Peaks: (1) 2-chlorobenzoic acid, (2) 2-chlorobenzamide, (3) (*p*-chlorophenyl)urea, (4) *p*-chloroaniline.

CCU were 5.0, 8.5, 11.3, 13.3, and 38.1 min, respectively. CCU, 2-chlorobenzoic acid, and 2-chlorobenzamide were quantified with an external standard method. The results were as follows: The concentrations of CCU, 2-chlorobenzoic acid, and 2-chlorobenzamide were 1.41×10^{-2}, 1.28×10^{-3}, and 1.16×10^{-4} mmol/L, respectively.

In this case, the values of a, t, and y were 1.55×10^{-2} mmol/L, 30 h, and 1.16×10^{-4} mmol/L, respectively. From **Table 1**, the value of $k_1 + k_2$ (namely, k) at pH 6 can be obtained as 3.29×10^{-3} h^{-1}. Further substituting the according values of a, y, t, and k into eq 6, the k_2 value was calculated as 2.68×10^{-4} h^{-1}.

3.2.4. Prediction of y_m. The values of $k_1 + k_2$ (namely, k) and k_3 at pH 6 were obtained as 3.29×10^{-3} and 2.86×10^{-3} h^{-1}. The value of k_2 was determined as 2.68×10^{-4} h^{-1} in section 3.2.3. Substituting these values of $k_1 + k_2$, k_3, and k_2 into eq 8, y_m was calculated as (3.2%)a. Therefore, the maximum concentration of 2-chlorobenzamide produced from CCU hydrolysis could reach 3.2% of the original concentration of CCU in pH 6 buffer solution.

3.3. Verification and Discussion of Predicted Results. *3.3.1. Verification*. The prediction model has been established, and the level and time of occurrence of the peak concentration of 2-chlorobenzamide were also achieved. Yet, whether it is valid or not still needs further confirmation. To verify the prediction model, another experiment was performed.

A 0.5 mL sample of CCU stock solution was diluted to 5 mL with methanol and then transferred into buffer solution (pH 6), and the terminal volume was controlled as 300 mL. The hydrolysis occurred with keeping the solution in the thermostatic water bath and avoiding light at 25 °C. Every 5 d, duplicate samples were taken out for analyzing the content of CCU and 2-chlorobenzamide on HPLC, and the results are shown in **Figure 3**.

From **Figure 3**, the hydrolytic half-life of CCU was about 8 d, which was close to the reported 8.78 d (*12*). And the 2-chlorobenzamide reached the peak concentration near the 22nd day, and the peak concentration was about 1.6% of the original concentration of CCU.

In the previous section, predicted values of the time of occurrence of the peak concentration and the level of 2-chlorobenzamide were 13.5 d and 3.2% of the initial concentration of CCU in pH 6 water buffer solution without light. The predicted values are similar to the experimental data.

3.3.2. Discussion. Although the predicted values were close to the experimental data, there was a difference between the predicted values and experimental data. The predicted peak

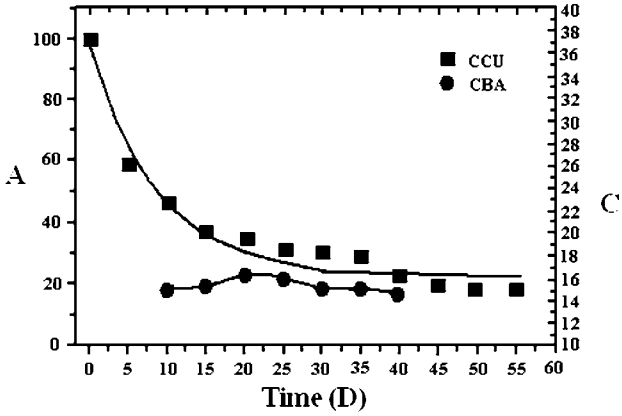


Figure 3. Formation of 2-chlorobenzamide in the hydrolysis of CCU. A = ratio of CCU concentration to its initial concentration × 100. C = ratio of CBA concentration to the initial concentration of CCU × 1000.

occurrence time was earlier than the determined value, and the predicted peak concentration was also higher than the experimental values. What caused these results was that 2-chlorobenzamide was insoluble and the solution for hydrolysis was obtained by adding a stock methanol solution of 2-chlorobenzamide to water under ultrasonic conditions (*18*), and 2-chlorobenzamide was dispersed as microparticles in the water. In the verification experiment, 2-chlorobenzamide produced from the hydrolysis of CCU existed as a novel state and underwent hydrolysis immediately. The size of the particles would affect the hydrolytic rate; i.e., the larger the size, the smaller the hydrolytic rate. Therefore, the hydrolytic rate of 2-chlorobenzamide (k_3) in ref *18* is smaller than the verification experimental data. Thus, the hydrolytic rate (k_3) used in the prediction model from ref *18* was smaller than the real hydrolytic rate of 2-chlorobenzamide produced in the hydrolysis of CCU. The smaller k_3 used in eqs 7 and 8 caused the predication t_m to be smaller and y_m to be bigger than those obtained from experiment. If we were to measure the hydrolytic rate of 2-chlorobenzamide (k_3) more accurately, it would be expected that the prediction values would be more coincident with the real values. On a whole, the peak concentration and the time of occurrence of the peak concentration from the proposed prediction model could match very well those from the real conditions, so the prediction model was credible.

3.4. Conclusion. 2-Chlorobenzamide has been known as a potential carcinogen and also one of the main CCU decomposition products in the environment. Due to the low toxic effect of CCU on organisms, 2-chlorobenzamide generated from the hydrolysis of CCU has been an environmentally concerning compound and has played an important role in the environmental safety of CCU. The peak concentration of 2-chlorobenzamide and the time of occurrence had been related to the level of the environmental danger of CCU. A simple, rapid, and convenient prediction model was established for prediction of the peak concentration and the time of occurrence of the peak concentration of 2-chlorobenzamide in the hydrolysis of CCU in this study. Verification experiments showed that the predicted results matched the real analytical data closely. Therefore, the proposed prediction model was highly credible and could be used as an effective tool to predict the concentration of 2-chlorobenzamide when CCU hydrolyzes in the real environment, related to the environmental safety of CCU.

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