

Photodegradation of 1-(2-Chlorobenzoyl)-3-(4-chlorophenyl) Urea in Different Media and Toxicity of Its Reaction Products

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The photodegradation of 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea in simulated air, methanol, dioxane, hexane, and water, with a xenon lamp as light source, was studied. The rate constants and half-lives of this compound in various media under nitrogen or oxygen were determined. The photoreaction products were analyzed with HPLC-UV, GC-MS, and direct probe MS and found to have some differences in different cases. With ³²P postlabeling DNA adduct formation experiments, one of the main products, 2-chlorobenzamide, was found to be able to form a DNA adduct.

Keywords: 1-(2-Chlorobenzoyl)-3-(4-chlorophenyl) urea; photodegradation; 2-chlorobenzamide; DNA adducts

1. INTRODUCTION

1-(2-Chlorobenzoyl)-3-(4-chlorophenyl) urea (CCU), also named benzamide [2-chloro-(((4-chlorophenyl)-amino)carbonyl)], is a new chemical of phenylurea insecticides, after DFB and PH6038. It has been synthesized and commercialized in China since the early 1980s and is now used widely in forestry and agriculture. Some physicochemical properties of CCU and also its IR, UV, and MS spectra had been reported in one of our previous papers (1). Its melting point was determined as 196–198 °C, and thermogravimetric analysis indicates that it is stable below 230 °C. In this paper, our results of the kinetics and mechanisms of the photodegradation of CCU in simulated air, in organic solvents, and in water will be reported and discussed. These results can serve as supplemental data for the safety evaluation of the environmental effects of CCU.

2. MATERIALS AND METHODS

2.1. Materials. CCU was produced by Tonghua Factory of Insecticides (Jinlin Province), and after recrystallization three times with toluene, its purity was ~98%. Silica gel (100–200 mesh) was from Shanghai Wusi Chemical Factory and was pretreated with methanol and dichloromethane before use (1). All solvents, methanol, 1,4-dioxane, and dichloromethane, were from Beijing Chemical Factory and redistilled before use. Four standard compounds, 4-chloroaniline, 4-chlorophenyl urea, 2-chlorobenzamide, and 2-chlorobenzoic acid, were supplied by Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. Chlorobenzene was the standard reagent from the U.S. EPA. 2-Chloro-*N*-(4-chlorophenyl) benzamide was from an HPLC fraction and identified with MS.

2.2. Apparatus. A Shimadzu LC-5A HPLC was equipped with an SPD-1 UV detector, the GC-MS was a Finnigan MAT-4510, and a Branson B-32 ultrasonicator was used. As the radiating spectrum of xenon lamp continues from UV to near-

IR, which is similar to sunlight (2, 3), an XQ-200 W xenon lamp with XQ150-500 DC power from Shanghai Lightening Device Factory was taken as light source in all of our experiment. A 75L-III accumulation emission meter from Tianjin Third Analytical Instrument Factory was used to measure the light intensity in the reactor. The photoreaction instruments were described elsewhere (4–7), with quartz reactors made in our center.

2.3. Procedures. **2.3.1. Photoreaction Experiment.** **2.3.1.1. Under Simulated Atmospheric Condition.** CCU was coated on silica gel (4). Approximately 1 g of the silica gel sample was introduced into the fluidized bed quartz reaction chamber and suspended in oxygen or nitrogen, with the flow rate controlled at 100 mL/min. The xenon lamp was then turned on, and the light intensity in the reactor was measured as 2.60 J/cm²·min. During irradiation, sample aliquots of ~100 mg each were taken out at set time intervals and then weighed and extracted with dioxane. Thus, the variation of concentration of CCU on silica gel was measured with HPLC. After 120 h of exposure, the sample was taken from the reaction chamber and extracted with methanol. The extract solution was concentrated to 1 mL, and the reaction products were identified with HPLC-UV, GC-MS, and direct probe MS.

2.3.1.2. In Organic Solvents. Fifty milliliters of methanol, dioxane, or hexane solution at ~50 ppm of CCU was added into the quartz cylindrical reaction chamber (25 ± 2 °C), and nitrogen or oxygen was passed through the solution at 40 mL/min to keep the solution saturated. The variation of the CCU concentration with time of exposure under the xenon lamp, the light intensity in the reactor measured as 2.10 J/cm²·min, was studied by taking samples at set time intervals and analyzing with HPLC. After 15 h, the solution was transferred to the K-D concentrator and concentrated to 1 mL. The products were then identified with HPLC-UV and GC-MS.

2.3.1.3. In Water Phase. Five hundred milliliters of water solution with CCU at ~5 ppm was added into a cool photoreactor, and oxygen or nitrogen was passed through the solution at 400 mL/min. At every fixed time interval, 5 mL of solution was taken out from the reactor and extracted with dichloromethane. After concentration, the extract was dissolved in 0.5 mL of methanol and analyzed with HPLC (7). After 30 h of exposure, all of the solution was taken out and extracted, the extract after concentration was dissolved in 0.1 mL of methanol, and the photoproducts were identified with HPLC-UV and GC-MS.

Meanwhile, dark experiments under the same conditions and with the same procedures as mentioned above for the

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Table 1. Net Photoreaction Kinetics Equations

medium	gas	equation	R	half-life (h)
simulated atmosphere	O ₂	1/c = 0.295 + 0.00445t	0.983	62.7
	N ₂	ln 1/c = -1.27 + 0.00703t	0.989	98.6
methanol	O ₂	ln 1/c = -3.99 + 0.129t	0.998	5.37
	N ₂	ln 1/c = -3.98 + 0.118t	0.997	5.87
1,4-dioxane	O ₂	ln 1/c = -3.94 + 0.182t	0.999	3.81
	N ₂	ln 1/c = -3.98 + 0.278t	0.997	2.49
hexane	O ₂	ln 1/c = -2.86 + 1.70t	0.999	0.41
	N ₂	ln 1/c = -2.87 + 1.69t	0.997	0.41
water	O ₂	ln c/c ₀ = -0.0227t		31
	N ₂	ln c/c ₀ = -0.0125t		55

different cases were carried out in parallel for comparison without the application of the xenon lamp.

2.3.2. DNA Adduct Formation Experiment. Both the mixture of products and 2-chlorobenzamide (one of the main products of CCU) were tested with ³²P-postlabeling for DNA adduct formation, following the procedure described (8).

2.4. HPLC Analysis. HPLC was used to determine the concentration of CCU during experiments. The chromatographic conditions used were as follows: HPLC column, 250 × 4.6 mm Zorbax C8; mobile phase, CH₃OH/H₂O (90:10 v/v), at a flow rate of 1 mL/min; detector, UV-254 nm.

2.5. Identification of the Reaction Products. **2.5.1. Separation and Identification by HPLC.** The chromatographic conditions used were as follows: HPLC column, 250 × 4.6 mm Zorbax C8; mobile phase, CH₃OH/H₂O (70:30 v/v), at a flow rate of 0.4 mL/min; detector, UV-254 nm.

2.5.2 Identification with GC-MS. GC/MS conditions were as follows: SE54 capillary fused silica column of 30 m × i.d., 0.22 mm; column temperature programmed from 60 to 220 °C at 4 °C/min and then from 220 to 300 °C at 10 °C/min; MS system with electron impact mode at 70 eV, ion current at 270 μA, mass scan range from 33 to 500 amu, with scan rate at 30 amu/min and ionization temperature at 300 °C.

2.5.3. Identification with Direct Probe MS. The reaction products were separately collected with HPLC and then identified with direct probe MS. The MS condition was an ionization temperature gradient from 80 to 300 °C at 30 °C/min.

3. RESULTS AND DISCUSSION

3.1. Photodegradation Kinetics. The apparent photodegradation and the dark reaction of CCU under simulated atmospheric condition, in organic solvents and water phase, and under oxygen or nitrogen were studied in parallel. The results showed that the dark reactions are unimportant under simulated atmospheric condition or in organic solvents, whereas the dark reaction in water is important. These results imply that CCU is stable in the particle-bound form or in organic solvents under dark conditions, but hydrolysis occurs in water phase no matter whether the light exists or not.

The photodegradation kinetics are almost all of first order, except that the photoreaction under simulated atmospheric condition with oxygen as saturating gas is of second order. The net photodegradation kinetics equations are listed in Table 1.

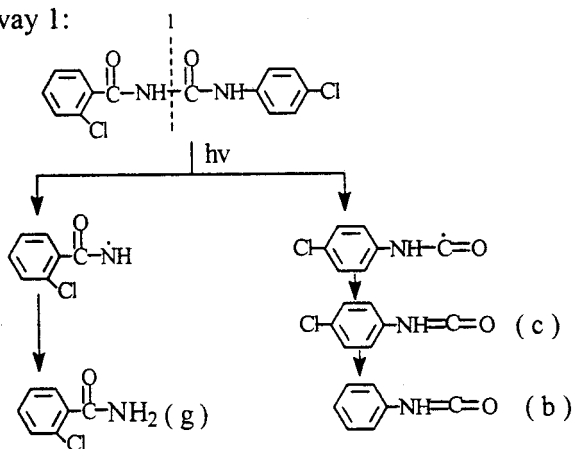
From Table 1, it can be seen that photoreactions are faster in simulated atmosphere, 1,4-dioxane, or water when oxygen is used as the saturating gas instead of nitrogen. However, there are no differences in methanol and hexane when either of these two saturating gases is used. The reaction rates for different media follow the order hexane > dioxane > methanol > water.

3.2. Photodegradation Products. The photodegradation products of CCU in various media identified with HPLC-UV, GC-MS, and direct probe MS are listed in Table 2.

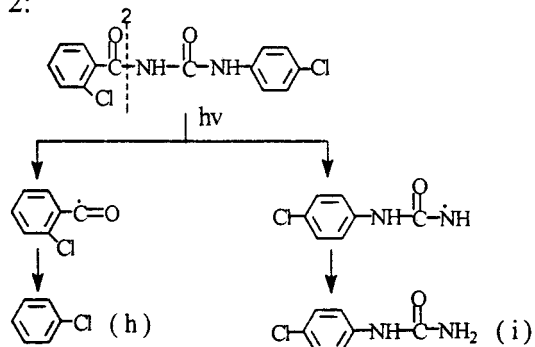
2-Chlorobenzamide is always the main photoproduct in every case; the relative amounts of 4-chlorophenyl isocyanate and its derivative *N*-(4-chlorophenyl) methylcarbamate or *N*-phenyl methylcarbamate are high or medium in different media.

3.3. Mechanism of Photodegradation. The photodegradation rates of CCU in different media are ordered as follows: in hexane > in dioxane > in methanol > in water, with the increasing polarity of these solvent media. This means that the photodegradation of CCU probably or most likely occurs through free radical pathways. On the basis of the chemical identities of the products, the mechanism of photodegradation is suggested as follows:

Pathway 1:



Pathway 2:



Pathway 3:

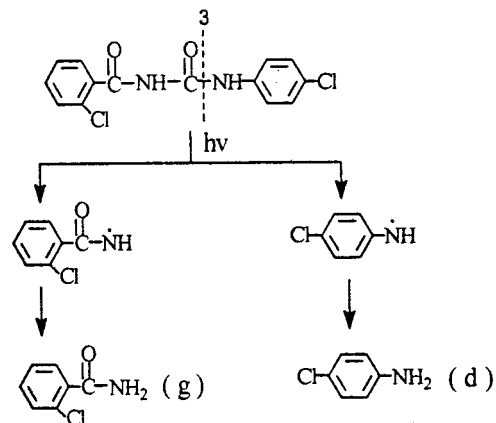


Table 2. Photodegradation Products

Number	Structure formula	Identified by **	RT(min)		Mol.wt	Main MS fragm	Relative content in various media *				
			HPLC	GC-MS			Simulated atmosphere	methanol	dioxane	hexane	water
a		1	5.0		156	-					H
b		2		7.7	119	119(M ⁺), 91		L			
c		2		13.8	153	155, 153(M ⁺), 125, 90	M	L	H		H
d		1,2	11.0	18.4	127	129, 127(M ⁺), 92	M				L
e		2		20.8	170	172, 170(M ⁺), 139, 111	L				
f		2		21.6	151	151(M ⁺), 119, 92, 77		H			
g		1,2	7.5	26.2	155	157, 155(M ⁺), 139, 111	H	H	H	H	H
h		1	9.3		112	-		L			
i		1,3	9.8		170	172, 170(M ⁺), 127	M	L	H		H
j		2		28.4	185	187, 185(M ⁺), 153, 126		M			H
k		2,3	18.8	46.4	265	267, 265(M ⁺), 139, 111	L	L			L

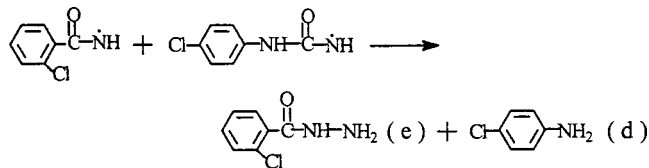
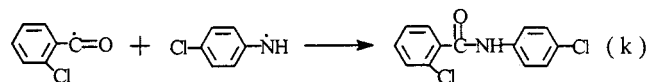
* H-high, M-medium, L-low. ** 1=HPLC-UV, the retention time in HPLC and the UV-spectrum of the product was as the same as that of standard compound. 2=GC-MS the products were identified with GC-MS. 3= Direct Probe MS the products were separately collected with HPLC, and then identified with direct probe MS.

Table 3. Relative Adductive Rates of DNA Adducts

chemical	spot 1	spot 2	spot 3/3	spot 4
products mixture		7.1×10^{-9} RAL ^a	2.3×10^{-8} RAL	8.0×10^{-8} RAL
CBA	1.1×10^{-8} RAL	4.1×10^{-9} RAL	3.9×10^{-9} RAL	3.0×10^{-8} RAL

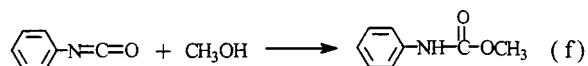
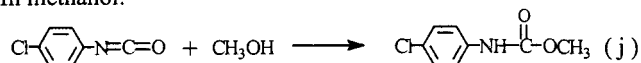
^a RAL, relative adduct labeling = [radioactive count of adducted base (cpm) - blank radioactive count (cpm)] ÷ [radioactive count of normal DNA base (cpm) + Σ radioactive count of adducted base (cpm)].

Some free radicals can react with each other to form some new products, such as

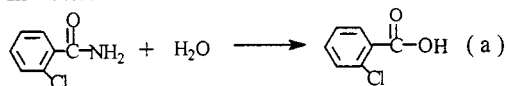


Moreover, some products can further react with solvent molecules as follows:

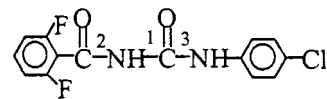
In methanol:



In water:



According to Riley (9), the lengths of amide bonds 1, 2, and 3 in DFB are 1.410, 1.378, and 1.337 Å,



respectively. The stability sequence of these three bond is $1 < 2 < 3$. Considering the similar structures of CCU and DFB, we could infer that pathway 1 possibly is the easiest to take place, whereas pathway 2 or 3 is more difficult to take place. Thus, 2-chlorobenzamide, 4-chlorophenyl isocyanate, isocyanatobenzene, and their derivatives should be the main products. This is consistent with the results of our experiments.

3.4. DNA Adducts. With the ³²P postlabeling test, both the product mixture of CCU and 2-chlorobenzamide (CBA), which is one of the main products, are found to be able to react with DNA to form similar adducts. The results can be seen from Figure 1.

From Figure 1, three and four visible spots of DNA adducts with the mixture of degradation products and CBA, respectively, can be seen, and their relative adductive rates are shown in Table 3.

The results from Figure 1 and Table 3 show that CBA is the main dedicator for DNA adduct formation in the product mixture.

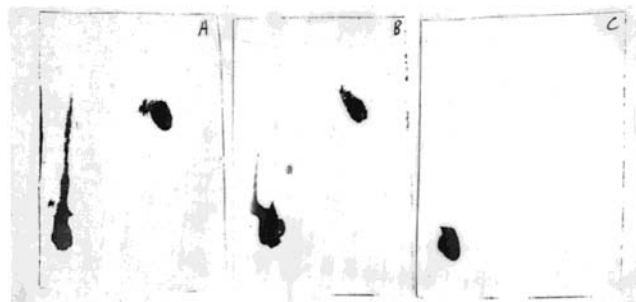


Figure 1. Autoradiograms of DNA adduct formation: (A) degradation products mixture of CCU-DNA adducts; (B) CBA-DNA adducts; (C) blank DNA (calf thymus).

If one chemical can form adducts with DNA, and the injured DNA cannot be repaired, the chemical is a potential mutagen, carcinogen, or teratogen. Accordingly, CBA may be a potential carcinogen or mutagen, especially due to the fact that a positive result for CBA in the Ames test has been noted by Dr. Wang Dai of the Institute of Biochemistry, Chinese Academy of Sciences. It is now suggested that for the safety evaluation of pesticides, the properties of not only the pesticide but also its products should be considered. As CBA is one of the main degradation products of CCU, the safety of CCU to ecological systems or human health should be of great concern. Further studies about CCU in the environment are being carried out in our laboratory (10, 11).

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LITERATURE CITED

- (1) Liu, Guoguang; Jin, Zuliang; Xu, Xiaobai. Preliminary study of the kinetics of photodecomposition of Benzamide, 2-chloro-*N*-(((4-chlorophenyl)amino)carbonyl) under simulated atmospheric conditions. *Huanjing Kexue* **1990**, *11* (5), 12–15.
- (2) Li, A. S. W.; Chignell, C. F. Spectroscopic studies of cutaneous photosensitizing agents—XI. Photolysis of chlorpromazine metabolites: a spin-trapping study. *Photochem. Photobiol.* **1987**, *45*, 695–701.

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- (3) Niu, E.; Ghiggino, K. P.; Mau, A. W. H.; Sasse W. H. F. Fluorescence and photochemistry of dye sensitizers in Nafion membrane. *J. Luminescence* **1988**, *40–41*, 563–564.
- (4) Liu, Guoguang; Jin, Zuliang; Xu, Xiaobai. Photoreaction of 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea in simulated atmosphere. *J. Environ. Sci. (China)* **1992**, *4* (4), 52–60.
- (5) Liu, Guoguang; Jin, Zuliang; Xu, Xiaobai. Study on the photoreaction of 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea in methanol. *J. Environ. Sci. (China)* **1992**, *4* (4), 61–71.
- (6) Liu, Guoguang. Study on the photoreaction of 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea. Doctoral Dissertation, Research Center for ECO-Environmental Sciences, Chinese Academy of Sciences, Beijing, China, 1990.
- (7) Liu, Guoguang; Xu, Xiaobai. The preliminary study of the kinetics of photodecomposition of Benzamide, 2-chloro-*N*-(((4-chlorophenyl)amino)carbonyl) in water. *Huanjing Huaxue* **1993**, *12* (1), 47–52.
- (8) Liu, Sufen; Jiang, Xiangning; Liu, Guoguang; Xu, Xiaobai. Investigation of DNA-2-chlorobenzamide adduct. *Huanjing Huaxue* **1997**, *16* (4), 316–320.
- (9) Riley, W. C.; Jacobson, R. A. 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea (Difluorbenuron). *Cryst. Struct. Commun.* **1981**, *10*, 1341.
- (10) Zhou, Qingxiang; Lu, Wenying; Liu, Guoguang; Xue, Xiuling. Study on the hydrolysis of 2-chlorobenzamide. *J. Agric. Food Chem.* **2000**, *48*, 2572–2575.
- (11) Liu, Guoguang; Zhou, Qingxiang; Lu, Wenying. The formation of 2-chlorobenzamide upon hydrolysis of the benzoylphenylurea. Insecticide 1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea in different water systems. *J. Agric. Food Chem.* **2001**, *49*, 1304–1308.

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